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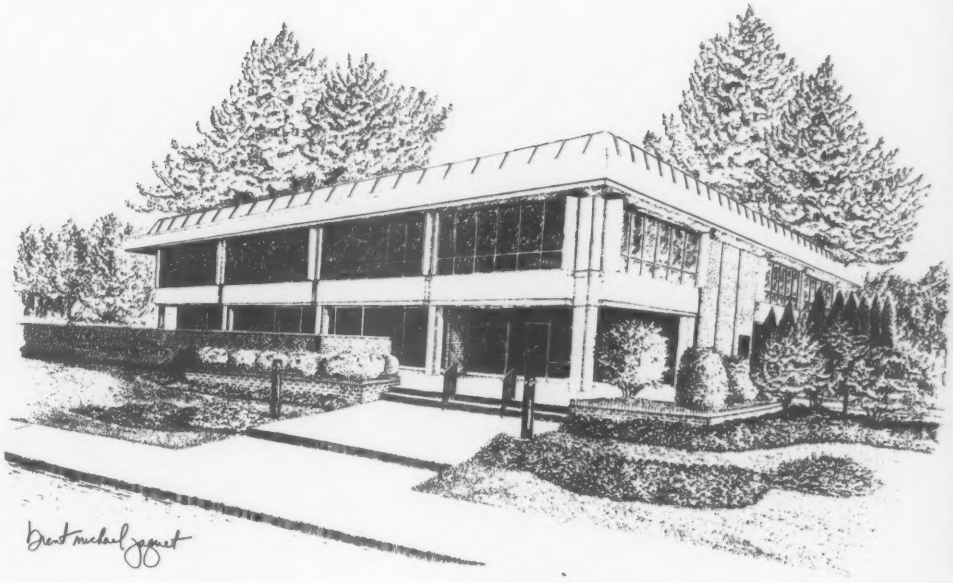

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COMMENTARY

Clinical Application of the Concept of Methotrexate Plus 5-FU Sequence-Dependent "Synergy": How Good Is the Evidence?[1]

George P. Browman[2,*]

The medical oncology community has enthusiastically embraced the concept of sequence-dependent synergy for the combination of methotrexate (MTX) and 5-FU. A recent symposium was entirely devoted to this issue (1). MTX-5-FU sequence-dependent synergy has been shown to occur in experimental tumor and immunologic systems in vitro and in vivo (2–9). However, the interpretations of the results of some of the in vivo experiments are open to question and will be discussed. In the wake of accumulating evidence for MTX-5-FU synergy in experimental tumor systems, there has been a proliferation of phase II clinical studies done primarily in breast, colon, and squamous cell head and neck cancers (1,10–21). The results, although promising in some studies, do not allow investigators to make claims regarding the clinical applicability of the synergy concept. Nevertheless, the MTX-5-FU synergy concept holds a prominent place in the design of future therapeutic strategies to the point of inclusion as adjuvant therapy in large trials for early-stage breast cancer.[3]

The purpose of this paper is to sound a note of caution regarding the premature routine use of this inadequately tested regimen. To do this, I will put forward arguments based on experimental evidence which suggest that the concept of sequence-dependent MTX-5-FU synergy needs more careful examination before it becomes more widely applied in the clinic.

Evidence From In Vivo Experimental Models

The concept of MTX-5-FU sequence-dependent synergy arose out of studies initially performed in in vivo immunologic models which demonstrated potentiation of suppression of humoral antibody production when MTX preceded 5-FU administration in mice (2,3). This sequence produced more profound suppression than schedules of simultaneous MTX plus 5-FU administration or when 5-FU preceded MTX. No effects were seen on tests of cell-mediated immune response. These initial studies, however, employed calcium leucovorin with MTX. This confounds interpretation of the mechanism responsible for the observed effects, since it has been shown that calcium leucovorin alone will enhance the antimetabolic effect of 5-FU (22–24).

The sequence-dependent nature of the cytotoxic effect of MTX and 5-FU has been studied in a number of experimental tumor models. Lee and Khwaja (4) demonstrated that following the removal of rat mammary carcinoma 13762, the adjuvant effect of chemotherapy with MTX-5-FU was maximal when MTX preceded 5-FU. However, the benefit was confined to animals with small initial tumors, while in those with large tumors, MTX pretreatment actually decreased the therapeutic effect obtained with 5-FU alone.

Studies carried out by Heppner and Calabresi (5) showed that the antitumor effect of the MTX-5-FU combination was maximal when MTX preceded 5-FU. However, these investigators also employed calcium leucovorin simultaneously with MTX so that in the sequence MTX → 5-FU, leucovorin also preceded 5-FU administration. As previously indicated, this confounds interpretation of the results, since leucovorin itself may enhance the effect of 5-FU (22–24).

In an elegant series of experiments with the mouse Sarcoma 180 model, Bertino et al (6) also demonstrated that the antitumor effect of the combination of MTX and 5-FU was maximal when MTX preceded 5-FU. Maximum benefit was reached with a 1-hour delay interval and was maintained for 4 hours. This remains the only in vivo study in an experimental system which conclusively demonstrates the synergy concept.

Biochemical Considerations

5-FU is thought to produce its cytotoxic effects by three possible mechanisms, all of which initially require "activation" of the drug to intracellular nucleotide metabolites. This intracellular activation may occur by two

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pathways. One pathway, which has been shown to be important in some experimental systems (25,26), involves the use of phosphoribosylpyrophosphate (PRPP) as a cosubstrate for the one-step conversion of 5-FU to the nucleotide FUMP via a reaction catalyzed by the enzyme orotate phosphoribosyltransferase. PRPP is an important early intermediary compound for de novo purine synthesis and intracellular PRPP can be elevated by agents, including MTX, that inhibit de novo purine synthesis (27,28). MTX-induced elevation of PRPP pools is also associated with enhanced 5-FU nucleotide formation and increased incorporation of labeled drug into RNA (8,28,29). This chain of events, which results in fraudulent RNA formation, represents one hypothesis to explain MTX-5-FU sequence-dependent synergy.

Others hypothesize that MTX + 5-FU sequence-dependent synergy may occur at the level of the target enzyme thymidylate synthetase. Inhibition of thymidylate synthetase is accomplished by the 5-FU metabolite 5-fluorodeoxyuridylate (FdUMP), which forms part of a ternary complex with the enzyme and with the cosubstrate 5,10-methylenetetrahydrofolic acid ($5,10CH_2$-FH_4). The availability of $-5,10CH_2$-FH_4 for complex formation is an important determinant of the degree of FdUMP inhibition of the enzyme (30). MTX will deplete reduced folate pools in DNA synthesizing cells. It has therefore been argued that MTX should antagonize the inhibitory effect of thymidylate synthetase by FdUMP through a decrease in reduced folate pools available for ternary complex formation. The fall in reduced folate pools, however, is accompanied by increased dihydrofolate levels, which Fernandes et al (31,32) have shown can substitute for $5,10CH_2$-FH_4 in ternary complex formation with FdUMP and thymidylate synthetase. Furthermore, it has been shown that MTX polyglutamates can take part in the formation of a stable ternary complex.

A third potential mechanism of MTX-5-FU synergy which has recently been invoked is based on observations that nucleotide metabolites of 5-fluoropyrimidines are incorporated into the DNA of treated cells (33–36). In the presence of MTX (and thymidine), there is enhanced excision of 5-FU residues from DNA, which could result in increased DNA fragmentation through cyclic incorporation and removal of 5-FU nucleotides by excision-repair processes (37,38). There is controversy as to whether sufficient incorporation of 5-FU nucleotides into DNA occurs to account for cytotoxicity (34). However, cytotoxicity may depend more on the frequency of misincorporation (insertion-excision) of 5-FU nucleotides into nucleic acids than on the absolute amount incorporated. Nevertheless, in terms of the MTX-5-FU interaction, the MTX-induced enhancement of excision of 5-FU residues from DNA was dependent on the presence of thymidine at concentrations far above measured physiologic levels (37,39).

Whether or not the experimental observations and theoretic hypotheses supporting sequence-dependent MTX-5-FU synergy are clinically applicable would depend on a number of other equally important hypothetic factors.

There are theoretic biochemical circumstances which can abrogate the proposed mechanisms of MTX-5-FU synergy:

(*a*) Despite the demonstration that MTX polyglutamates and dihydrofolate polyglutamates can participate in ternary complex formation with FdUMP and thymidylate synthetase to inhibit the enzyme, it remains to be seen whether changes in these metabolites can sufficiently compensate for the depletion in reducted folate pools. There is experimental evidence that the sequence MTX → 5-FU can be antagonistic (40).

(*b*) The two main hypotheses to explain synergy both depend on the induction of pronounced biochemical effects by MTX pretreatment. Since the question of synergy is not relevant where MTX or 5-FU alone can produce cytocidal effects, the requirement is that MTX produce biochemical perturbations sufficient to elevate PRPP and/or dihydrofolate levels but insufficient to kill cells. To enhance 5-FU incorporation into RNA requires inhibition of de novo purine biosynthesis so that PRPP will become elevated and enhance 5-FU anabolism to nucleotides. De novo purine biosynthesis is more resistant to MTX inhibition than thymidylate synthesis (41). Therefore, a further requirement is that cells be sensitive enough to MTX (ie, sensitive enough to folate depletion) so that both thymidylate and purine synthesis are inhibited, but not sufficiently sensitive so that cells will die. Furthermore, if the purine synthetic pathway is exquisitely sensitive to MTX, then it may be markedly inhibited, thus reducing RNA synthesis and possibly compromising the incorporation of 5-FU into RNA. It has been demonstrated in murine leukemia cells that preincubation with MTX at progressively higher concentrations results in a progressive increase of 5-FU incorporation into RNA. However, an optimal MTX concentration is reached, beyond which a further rise reduced 5-FU incorporation into RNA, presumably due to a decrease in the total amount of RNA synthesized (11). The logical extension of these arguments is that synergy would depend on administration of an optimal dose of MTX (ie, too low a dose, and the biochemical perturbations required for synergy do not occur; too high a dose, and RNA synthesis is inhibited to a point which makes synergy impossible or cell kill is achieved by MTX alone). Therefore, between the problems of optimal dose administration and cell sensitivity to MTX, there may be only a narrow window of an MTX dose range through which synergy can occur. Moreover, this window would likely vary among individuals. The ideal dose ranges and exposure times necessary to demonstrate synergy may be worked out in the laboratory, but may be difficult to achieve in the complex clinical situation.

(*c*) MTX-5-FU synergy will also depend on the relative importance of de novo and salvage routes for nucleic acid synthesis within tumor cell populations. The potential importance of these factors is made evident by experiments which showed that MTX pretreatment did not produce enhanced incorporation of labeled 5-FU into RNA in human bone marrow cells in vitro or mouse bone marrow in vivo where purines are synthesized primarily by the salvage route (11,26). The relative lack of de novo purine synthesis prevents buildup of PRPP levels on MTX exposure. Thus, anabolism of 5-FU to its nucleotide derivatives is not enhanced by this mechanism. Furthermore, hypoxanthine may compete for PRPP, thus reducing its availablility for 5-FU anabolism (42). In local areas of necrotic tissue and rapid cell turnover within tumors, purine salvage may play an important role and mitigate against MTX-5-FU synergy. Physiologic levels of the salvageable purine, hypoxanthine, are relatively high, vary considerably among individuals, have been found to be significantly increased in cancer patients, and may be as much as tenfold higher in local areas of rapid cell turnover (bone marrow) as compared with systemic levels within the same individual (43). The relevance of these considerations will not be known until we learn about the relative roles of salvage and de novo routes of nucleic acid metabolism in human tumors.

The availability of thymidine in areas of rapid cell turnover and tumor necrosis would allow bypass of the metabolic block produced by FdUMP and could prevent an MTX-induced depletion of reduced folates through negative feedback on the pathway catalyzed by thymidylate synthetase (44). This would not influence synergy to any greater extent than it would influence the effects of 5-FU or MTX alone; however, it does point out a circumstance where failure to respond to each of these drugs alone is unlikely to be overcome by a synergistic effect at the level of thymidylate synthetase. Moreover, thymidine availability may enhance the effect of 5-FU independent of MTX through increased incorporation of 5-FU nucleotides into RNA (45,46) or 5-FU deoxynucleotides into DNA (36).

(*d*) Cell kinetic considerations may determine whether or not MTX-5-FU synergy will occur and by what mechanism. In tumors with a large growth fraction, MTX is more likely to produce reduced folate depletion, since a large proportion of the cell population is synthesizing DNA. Thus, the biochemical perturbations produced by MTX will allow the changes needed to produce synergy. However, in tumors with a small growth fraction, a large proportion of cells will not be affected by MTX since, in the presence of MTX, DNA synthesis is required to produce a folate-depleted state. However, RNA synthesis must continue, even for cells not synthesizing DNA, so that structural and metabolic integrity can be maintained. Thus, although MTX may have little effect on most tumor cells, 5-FU can still produce cell death by incorporation into RNA. Studies have shown that 5-FU effects are not necessarily phase-specific and support a role for 5-FU incorporation into RNA as a mechanism of cytotoxicity (46,47). Further support comes from observations in vitro and in animals and man that administration of thymidine with 5-FU does not prevent toxicity as would be expected if the site of action of 5-FU were on thymidylate synthetase, but may enhance cytotoxicity by promoting increased 5-FU incorporation into RNA (45,46). Thus, in tumors with a small growth fraction, a situation exists where 5-FU may be active against a substantial proportion of the tumor cell population, but will be uninfluenced by the presence of MTX.

(*e*) Finally, the demonstration in the xenograft model that 5-FU activation to nucleotides can occur in human tumors via a pathway

$$\text{5-FU} \xrightarrow[\text{phosphorylase}]{\text{nucleoside}} \text{5-fluorouridine} \xrightarrow[\text{kinase}]{\text{nucleoside}} \text{5-fluorouridine}$$

monophosphate which is independent of changes in PRPP indicates that one major proposed mechanism for MTX-5-FU synergy may not be operative in a significant proportion of patients (26). In fact, studies of human colon cancer xenografts have failed to show an increase in therapeutic index for MTX-5-FU sequencing (48).

In addition to all of the above considerations, we must be aware of the potential for increased toxicity whenever metabolic modulation is employed. Although phase II studies have generally shown that MTX-5-FU sequencing is well-tolerated, there have been notable exceptions (12,49). Other attempts at metabolic modulation with antimetabolite combinations such as cytarabine and tetrahydrouridine (inhibits pyrimidine nucleoside deaminase, which inactivates cytarabine) and 6-mercaptopurine with allopurinol produced increased toxicity with little change in therapeutic index (50,51).

CONCLUSIONS

Investigators must be aware that there are valid theoretic biochemical considerations which should temper enthusiasm for the concept of MTX-5-FU synergy. Although sequence-dependent synergy can be demonstrated under carefully contrived laboratory conditions, our understanding of all the relevant biochemical events that occur in vivo is too rudimentary to allow us to draw conclusions about clinical efficacy. In vitro and in vivo evidence that the sequence can be antagonistic indicates that, in some patients, the treatment may be suboptimal. The evidence from experimental tumors demonstrating in vivo MTX-5-FU sequence-dependent synergy is controversial and, in some circumstances, difficult to interpret.

Therapeutic results from uncontrolled clinical studies of MTX-5-FU sequencing are conflicting, but give cause for encouragement, especially since responses were ob-

served in some patients known to be refractory to MTX + 5-FU used in the conventional fashion. In general, MTX-5-FU sequencing has been well-tolerated. In these respects, phase II trials have been useful. However, the results of two recently presented randomized trials failed to demonstrate an advantage for 1-hour sequential MTX-5-FU compared with simultaneous treatment in head and neck cancer or with the reverse sequence in head and neck, colon, and breast cancers (52-54). These results should not discourage further controlled randomized trials to test the sequencing concept. The issues on which these trials should focus are becoming clearly defined. These questions must be addressed in a variety of tumor types which should not be expected to behave similarly. On the basis of current evidence, however, clinical application of MTX-5-FU sequencing cannot be recommended except as part of appropriate studies.

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High-Dose Melphalan With Autologous Bone Marrow Rescue for the Treatment of Advanced Adult Solid Tumors[1]

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High-dose melphalan followed by "rescue" with autologous marrow stored for 12–24 hours at room temperature was used in the treatment of 14 patients with advanced tumors refractory to conventional treatment. Twelve patients were evaluable, with three complete responses (25%), five partial responses (42%), and two minimal responses (16%). Response durations ranged from 4 to 38 weeks (median, 7). There were two treatment-related deaths and one patient developed acute nonlymphocytic leukemia 3 months after a second course of high-dose melphalan. [Cancer Treat Rep 68:471–474, 1984]

The use of high-dose single or multiple-agent chemotherapy has recently been studied in patients with advanced solid tumors resistant to conventional chemotherapy (1–8).

We have performed a phase I–II study of high-dose melphalan (HDM) followed by the infusion of autologous bone marrow in the treatment of advanced solid tumors.

Melphalan was selected for this study because it has a high therapeutic index together with a broad spectrum of antitumor activity. It does not require metabolic activation and is rapidly excreted by the kidneys (9). Myelosuppression is its major toxic effect and, as has been shown by others (10), the period of hypoplasia can be effectively shortened by infusion of autologous marrow. The short half-life of iv melphalan (9) permits the infusion of fresh bone marrow without the need for cryopreservation.

PATIENTS AND METHODS

Patients.—Fourteen patients with progressive metastatic solid tumors resistant to conventional therapy were studied. Clinical data and prior radiation therapy are shown in table 1; all patients had progression of metastatic disease after conventional chemotherapy, with a median of six different major drugs (range, one to eight) given alone or in combination. Median time from initial diagnosis to HDM was 19.5 months (range, 4–292), and median time from onset of the metastatic disease to HDM was 9.5 months (range, 2–41). All patients had normal isotope bone scans, and no patient had evidence of tumor involvement of the marrow in multiple bone marrow biopsy specimens. Other baseline investigations included cbc, biochemical profile including liver and kidney function tests, chest roentgenogram, and electrocardiogram. Other tests such as ultrasound and computerized tomographic scans were also done, when needed, to assess the extent of disease.

Marrow harvest.—Under general anesthesia, bone marrow cells were taken from the anterior and posterior iliac crests of each patient and collected into sterile plastic bags containing tissue culture medium (RPMI-1640) and preservative-free heparin. Median yield of nucleated marrow cells was 1.86×10^8/kg (range, 0.5–3.5). Fat was removed by centrifuging the marrow suspension, which was then kept at room temperature for 12 hours (seven patients) to 24 hours (seven patients) before being returned to the patient. Room temperature was preferred to 4°C to store marrow for 24 hours because, in our experimental conditions, the recovery of colony-forming units (CFU-C) was better. In four experiments, the mean numbers (± SEM) of CFU-C/2 × 10^5 nucleated cells were 24 ± 2 at the time of marrow aspiration versus 16 ± 4 when marrow was kept at 4°C and 22 ± 8 when marrow was kept at room temperature for 24 hours.

Chemotherapy administration and dosage.—Melphalan was given by iv bolus through a central venous line previously inserted. Eleven patients received 140 mg/m² and three received 240 mg/m². All patients were well-hydrat-

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TABLE 1.—Patient data and response to HDM

Patient No.	Diagnosis and disease extension	Karnofsky performance status (%)	Patient sex/age (yrs)	Prior therapy* and duration (mos) of metastatic disease	Response†	Response duration (wks)	Survival after HDM (wks)
1	Soft tissue sarcoma (malignant fibrous histiocytoma), with multiple subcutaneous nodules	60	F/60	RT, VCR, Cy, ADR, 5FU, Cis-pl (16)	CR	38	62
2	Soft tissue sarcoma (angiosarcoma) metastatic to lungs, pleura, and lymph nodes	30	M/35	RT, ADR, VCR, DTIC, Cy (2)	MR	4	8
3	Breast carcinoma metastatic to lungs, pleura, skin, and liver	40	F/35	RT, MLP, VCR, ADR, CY, 5FU, Cis-pl (41)	NE	—	3
4	Breast carcinoma metastatic to lungs, skin, and lymph nodes	40	F/35	RT, ADR, Cy, VCR, 5FU, VDS (19)	NE	—	3
5	Ovarian carcinoma, disseminated abdominal disease	40	F/46	RT, Cis-pl, Cy, ADR, VCR (9)	CR	5	20
6	Uterine leiomyosarcoma metastatic to lungs, liver, and kidney	60	F/43	ADR, DTIC, VCR, Cy, VM26, VBL, Cis-pl, ACT-D (16)	PR	8+‡	55
7	Testis embryonal cell cancer metastatic to lymph nodes and lungs	90	M/41	RT, VBL, Cis-pl, VCR, Cy, ACT-D, ADR, BLEO (19)	PR	8+‡	28
8	Testis Sertoli cell cancer metastatic to skin, bones, and lymph nodes	70	M/41	VCR, Cy, ADR, Cis-pl, VBL, BLEO, ACT-D (8)	CR	8+‡	28
9	Primary mediastinal germ cell tumor metastatic to lungs and lymph nodes	60	M/37	ADR, VCR, Cy, VBL, BLEO, Cis-pl, ACT-D, RT (7)	MR	6	16
10	Primary mediastinal seminoma metastatic to lungs and liver	60	M/37	RT, ADR, VM26, Cis-pl, Cy, BLEO (3)	PR	6	20
11	Malignant thymoma metastatic to lungs, liver, and subcutaneous tissue	60	F/27	RT, VM26, Cis-pl (3)	PR	4	6
12§	Soft tissue sarcoma (synovial cell sarcoma) metastatic to lungs	70	F/28	ADR, VM26, DTIC, VCR, MTX, Cis-pl, BLEO (10)	SD	14	28
13§	Ovarian carcinoma, disseminated abdominal disease	40	F/57	ADR, VCR, 5FU, Cis-pl, THIOTEPA, HEXA (12)	NR	—	12
14§	Malignant melanoma, with multiple subcutaneous and lung metastasis	90	F/41	DTIC (7)	PR	11+‡	25

*RT = radiation therapy; VCR = vincristine; Cy = cyclophosphamide; ADR = doxorubicin; 5FU = 5-FU; Cis-pl = cisplatin; DTIC = dacarbazine; MLP = melphalan; VDS = vindesine; VM26 = teniposide; VBL = vinblastine; ACT-D = dactinomycin; BLEO = bleomycin; MTX = methotrexate; and HEXA = hexamethylmelamine.

†CR = complete response; PR = partial response; MR = minimal response; SD = stable disease; NR = no response; and NE = not evaluable.

‡These patients were given a 2nd course of HDM, 6–8 wks after the 1st course. All were still responding at the time of the 2nd treatment.

§Patients received 240 mg/m^2 iv of melphalan. All other patients received 140 mg/m^2 iv of melphalan.

ed with continuous iv fluids started 6-12 hours before and continued for 24 hours after HDM. Iv furosemide (20 mg) was given 1 hour after melphalan to induce a rapid diuresis.

Supportive care.—All patients had large-diameter right atrial catheters inserted upon admission, and most received iv alimentation (11). Three patients were treated in laminar air flow rooms and received oral prophylactic nonabsorbable antibiotics (tobramycin, colistin, vancomycin, and nystatin). Eleven patients were treated in single rooms with reverse-barrier nursing and received either prophylactic granulocyte transfusions or trimethoprim/sulfamethoxazole. Febrile episodes were promptly treated with broad-spectrum iv antibiotics. Random or single-donor platelets were given to maintain platelet counts $\geq 15 \times 10^9$/L. Rbc concentrates were given to maintain hemoglobin ≥ 10 g/dl. All blood products, except bone marrow, were irradiated with 1500 rads prior to transfusion.

Criteria for toxicity to the oral mucosa and the gastrointestinal tract.—The severity of mucositis was established using the following criteria: none = no pain; mild = discrete pain, able to eat; moderate = painful ulcerations, unable to eat most foods; and severe = pain requiring narcotics, unable to eat. The severity of diarrhea was evaluated as follows: none = normal stools; mild = watery stools $\leq$ 1000 ml/day for $<$ 3 days; moderate = watery stools $\leq$ 1000 ml/day lasting between 3 and 7 days or $>$ 1000 ml/day for $<$ 3 days; and severe = watery stools $\geq$ 1000 ml/day for $>$ 3 days or hemorrhagic enterocolitis.

Response to therapy.—The criteria for evaluation of response were as follows: *complete response (CR)*—the complete disappearance of all evidence of tumor at all sites of tumor involvement for ≥ 4 weeks; *partial response (PR)*—at least 50% diminution of tumor size lasting ≥ 4 weeks, without the appearance of new lesions; *minimal response*—a decrease of $<$ 50% in measurable lesions persisting for ≥ 4 weeks, without the appearance of new lesions; and *stable disease*—no evidence of disease progression for ≥ 4 weeks.

RESULTS

Toxicity

Profound myelosuppression occurred in all patients. Hematologic parameters tended to drop precipitously, with the median wbc count nadir on Day 8 (range, 5-11) and the median platelet count nadir on Day 11 (range, 6-16). Neutrophil counts were $< 0.5 \times 10^9$/L, for a median of 8 days (range, 5-23), and platelet counts were $< 20 \times 10^9$/L, for a median of 15 days (range, 7-23). Recovery of hematologic elements generally occurred by 4-6 weeks (table 2).

Patients were hospitalized for an average of 32 days. Retrospectively, we question the need for measures such as protected environment, since the duration of profound neutropenia with granulocyte counts $< 0.1 \times 10^9$/L was always $<$ 10 days (12).

All patients developed fever during neutropenia. Eight patients had documented bacterial infections and six had viral infections. Of these six episodes, four were the reactivation of mucocutaneous herpes simplex, one was viral hepatitis, and one was herpes encephalitis, which caused one early death on Day 18 (Patient 3).

There was one episode of acute cardiorespiratory failure, with dyspnea, tachycardia, and diffuse pulmonary interstitial infiltrates, which caused the other therapy-related early death on Day 17 (Patient 4). Respiratory distress occurred suddenly on Day 16, in the absence of documented infection (except herpes simplex stomatitis), and was marked by rapid and progressive congestive heart failure. The patient had received prior melphalan

TABLE 2.—Hematologic toxicity

Patient No.	Day of —		No. of days after transplant to achieve—	
	Leukocyte count nadir ($\leq 0.2 \times 10^9$/L)	Platelet count nadir ($\leq 20 \times 10^9$/L)	Neutrophil count $\geq 1 \times 10^9$/L	Platelet count $\geq 100 \times 10^9$/L
1	11	10	28	46
2	5	6	15	—
3	6	8	—	—
4	10	14	—	—
5	6	8	16	28
6	11	16	16	27
7	8	10	29	27
8	9	12	35	36
9	7	16	23	46
10	9	11	19	26
11	8	13	23	22
12	5	11	25	40
13	6	8	61	—
14	6	10	20	36

therapy and had a history of chronic respiratory insufficiency due to diffuse multinodular metastatic infiltrates. Gastrointestinal toxic effects included mild to moderate nausea and vomiting in all patients, moderate to severe mucositis in eight patients, and moderate to severe diarrhea in five patients. Similar gastrointestinal side effects were clinically noted to occur in the three patients treated with 240 mg/m^2 of melphalan.

No significant renal toxicity was observed. Alopecia occurred in all patients. Four patients received a second course of melphalan at the same dose 2 months after the first treatment, again in conjunction with autologous marrow rescue. Gastrointestinal toxicity was the same as after the first treatment in each patient. Marrow recovery was longer than after the first transplant, especially for platelets; in the four patients, neutropenia $\leq 0.5 \times 10^9$/L lasted a median of 16 days (range, 11-21) after the first transplant and a median of 18 days (range, 14-28) after the second; thrombocytopenia $\leq 20 \times 10^9$/L lasted a median of 12 days (range, 10-20) after the first transplant and a median of 31 days (range, 11-43) after the second.

Patient 8 developed acute myeloid leukemia 3 months after the second course. It followed a fulminant course, and the patient died within a few days of the diagnosis, having refused any further treatment.

Tumor Response

The two patients who died < 30 days after HDM were not evaluable for tumor response. The individual responses, doses of melphalan, response durations, and survival times are shown in table 1. Of the 12 evaluable patients, eight had objective responses (CR + PR), two had minimal responses, one had stable disease, and one had no response. Overall, the CR rate was 25% (three responses among 12 patients), the PR rate was 42% (five responses), and the minimal response rate was 16% (two responses).

DISCUSSION

All patients reported here had widespread metastatic disease which had progressed after conventional treatment. The overall response rate, with evidence of activity in 83% and objective evidence of response in 67% of the patients treated with HDM, compares favorably with other studies of single or combination high-dose chemotherapy (2-8). As noted by others (10), we found that fresh autologous marrow rescue was able to shorten the period of profound aplasia. Despite the poor performance status of many of our patients and the extensive prior chemotherapy, there were only two treatment-related deaths; patients as old as 57 and 60 years of age tolerated this therapy without untoward event. In the patient who developed leukemia (Patient 8), defective immune surveillance could have been responsible for the rapid proliferation of a leukemic clone, presumably emerging as a consequence of the leukemogenic effects of previous chemotherapy.

Despite the good overall response rates, response durations were short, ranging from 4 to 38 weeks.

Our findings are similar to those of other investigators (1-8) studying patients with advanced malignancies. We suggest that better results will probably be obtained if high-dose chemotherapy combined with autologous marrow rescue is given earlier in the course of the malignant disease. However, the potential risk of inducing a second malignancy will have to be taken into consideration.

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An Active Chemotherapy Regimen for Squamous Cell Lung Cancer[1]

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A new concept of chemotherapy scheduling was evaluated in 20 patients with inoperable squamous cell lung cancer. The complete plus partial response rate was 85%, with 15% complete responses. The drugs utilized included vinblastine, bleomycin, methotrexate, 5-FU, cisplatin, and leucovorin. The hypothesis leading to the chosen drug schedule was that impaired rbc deformability (RBCD) found in cancer patients may produce stasis of flow in tumor capillary beds and decrease drug delivery to cancer cells. Drug schedules were designed to take advantage of chemotherapy-induced improvement in RBCD. After an initial drug treatment, a second treatment was given when RBCD increased at least 25% over pretreatment values (usually 4–6 days after the first chemotherapy dose). Drug doses were weighted so that more drug was given when RBCD was greatest. Treatment toxicity was predominantly hematopoietic and renal. This type of chemotherapy approach opens up new avenues of investigation in squamous cell cancer and other common neoplasms. [Cancer Treat Rep 68:475–479, 1984]

Squamous cell lung cancer is considered to be a chemotherapy-resistant neoplasm. Despite partial response rates of 30% to > 40% in single institution trials, few complete responses have been achieved and prolongation of survival, beyond that expected for patients with favorable pretreatment prognostic factors, has not been convincingly demonstrated (1,2). New therapeutic approaches are necessary.

A hypothesis that has guided clinical and experimental studies by our group in recent years is that one cause of poor chemotherapy results may be the inability of cytotoxic drugs to reach tumor cells in sufficient concentration and for a sufficient time for optimal cell kill. It is widely appreciated that severe disturbances of blood flow occur within tumor tissue (3). Because of insufficient neovascularization, tumors have a markedly reduced vascular space per unit of tissue volume. Further, since cancers grow in a relatively confined space, cellular expansion often results in vascular compression (3). Other factors impairing tumor microcirculation include increased plasma viscosity, secondary to increased synthesis of acute phase proteins (4,5) and coagulation system activation with possible intravascular thrombosis and/or platelet aggregation (6,7). The net result of such processes is that the tumor milieu is markedly ischemic and acidotic (3). These unphysiologic conditions may result in decreased rbc deformability (RBCD) (8). The adverse consequences of decreased RBCD on microcirculatory blood flow can be appreciated from the fact that rbcs have a diameter two to two and one-half times greater than the capillaries that they traverse (9,10). Abnormal RBCD, in advanced cancer, has been demonstrated experimentally in mice bearing L1210 leukemia and Lewis lung carcinoma (11) and has also been noted in humans with small cell and non-small cell bronchogenic carcinoma (12,13).

In experimental and clinical studies, it was found that improvement of abnormal RBCD could be achieved by the administration of cytotoxic chemotherapy. Depending on the drug regimen used, RBCD considerably improved 4–13 days after treatment (14,15). In the L1210 system, if a second chemotherapy dose was administered at the time of maximal RBCD improvement, enhanced therapeutic results were achieved, with cure rates increasing from 0%–5% (in control animals) to 44% (15). In the current study, drug schedules were designed to take advantage of chemotherapy-induced improvement in RBCD. A treatment cycle consisted of an initial combination chemotherapy treatment followed by a second treatment when RBCD increased at least 25% over pretreat-

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ment values. Drug doses for each treatment cycle were weighted so that more drug was given when RBCD was greatest.

PATIENTS AND METHODS

Twenty male patients with squamous cell lung cancer who met protocol eligibility requirements have been treated. All patients had stage III disease according to American Joint Committee for Cancer Staging definitions (16). No patient had received prior chemotherapy or radiation therapy and no patient had documented brain metastases. All individuals were ambulatory for at least 50% of waking hours (Eastern Cooperative Oncology Group performance status ≤ 2) and had measurable or evaluable lesions on chest roentgenogram. Patients were required to have normal hematologic (wbc count $\geq 4000/mm^3$ and platelet count $\geq 100,000/mm^3$) and renal function (creatinine ≤ 1.5 mg/dl and creatinine clearance ≥ 65 ml/minute). All patients provided informed consent.

Initial staging evaluation consisted of complete history, physical examination, and laboratory evaluation, including a cbc, 18-channel biochemical profile, timed urine collection for creatinine determination, and chest roentgenogram (posteroanterior and lateral). All patients underwent fiberoptic bronchoscopy with cytology collection and with biopsies and/or brushings of all suspicious lesions. All patients had radionuclide bone, liver, and brain scans and computerized tomographic brain scans when clinically indicated. Follow-up studies included RBCD, cbc, and biochemical profiles performed twice weekly. Chest roentgenograms and tumor measurements were obtained weekly. Bronchoscopy with washing and biopsy was repeated in patients demonstrating a complete response on chest x-ray after four to six treatments. Abnormal pretreatment radionuclide scans were repeated prior to determination of patient response category.

The chemotherapy program is outlined in table 1. The drugs used were vinblastine, bleomycin, methotrexate, 5-FU, cisplatin, and leucovorin. All drugs were administered iv except leucovorin, which was administered either iv or orally. Vinblastine, bleomycin, and methotrexate were given by bolus injection into a freely running iv infusion. Four hours later, 5-FU (by bolus injection) and cisplatin (over 30 minutes) were administered. Leucovorin was started 20–24 hours after methotrexate and was given every 6 hours for four doses. Hydration generally consisted of 5% dextrose in 0.45% saline with 20 mEq of potassium chloride/L administered at a rate of 125 ml/hour for 24 hours. Following the second dose of cisplatin, in each treatment cycle, the iv infusion rate was increased to 200 ml/hour for 6 hours and the patient also received 20% mannitol at 50 ml/hour for 6 hours. Patients received acetaminophen (650 mg) prior to chemotherapy and were maintained on 140 to 280 mg of MgO orally daily throughout treatment. Antiemetics used in this study included droperidol, delta-9-tetrahydrocannabinol, and levonantradol. Chemotherapy cycles were repeated following hematologic recovery, generally 18–21 days after completion of the preceding treatment cycle.

After completion of two or three chemotherapy cycles (four to six chemotherapy doses), patients with disease initially encompassable within one radiation therapy port received a dose of 5000–5500 rad to the primary tumor and mediastinal and involved supraclavicular lymph nodes. Radiation therapy was given as a split course (a dose of 900 rad weekly for 7–8 weeks, with a 2-week rest period after one-half of the planned irradiation). The spinal cord was shielded after 4500 rad. After completion of radiation therapy, patients were followed, without additional therapy, to determine remission duration.

Complete and partial responses for measurable and evaluable disease were defined using standard criteria (17). A complete response was defined as the disappearance of all clinical evidence of disease, including negative bronchoscopic visual findings and cytologically negative washings and biopsies.

RBCD was measured by the method of Reid et al (*see* ref 18) with minor modifications. Venous blood was collected in 7-cm Vacutainer tubes containing sodium EDTA. After removal of plasma and a majority of the buffy coat, the rbcs were washed once with calcium- and magnesium-free phosphate-buffered saline (pH 7.4) containing 0.5% human serum albumin. After washing, a 1% rbc suspension was prepared in the above fluid. The washing procedure and RBCD determination were done at room temperature within 1–2 hours of blood collection.

The 1% rbc suspension in a 12-ml graduated vessel was filtered through a Nuclepore filter (3-μm pore diameter, 13-mm filter diameter) (Nuclepore Corporation, Pleasanton, CA). The negative filtration pressure was 20 cm of water. The filtered volume was determined at 1, 2, 4, 6, 8, and 10 minutes. RBCD was expressed as the total volume of rbc suspension filtered in 10 minutes (ml/10 minutes).

TABLE 1.—Squamous cell lung cancer treatment plan

Drug	Drug dose (mg/m^2)	
	Day 1	Day of increased RBCD (usually Day 4–6)
Vinblastine	4	6
Bleomycin	15	15
Methotrexate	60	60
5-FU	450–500*	600*
Cisplatin	20–30*	40*
Leucovorin	10 × 4 doses†	10 × 4 doses†

*Given 4 hrs after methotrexate.
†Started 20–24 hrs after methotrexate.

To determine the optimal time for a second chemotherapy treatment, RBCD was measured daily after the initial drug administration. The second chemotherapy treatment was given when RBCD had increased by at least 25% (arbitrary selection) over pretreatment levels. With the described drug regimen, increase in RBCD generally occurred between Days 4 and 6 (first day of treatment = Day 0). For patients with no increase in RBCD, a second dose of chemotherapy was given on Day 7 or 8.

RESULTS

Characteristics of the 20 male study patients are shown in table 2. Tumor size on chest roentgenogram decreased by > 50% in 17 patients (85%), including three who had complete roentgenographic disappearance of intrathoracic disease. Two of the latter three patients had repeat fiberoptic bronchoscopies after their second (Patient 3) and fourth (Patient 8) chemotherapy doses. Bronchial visual findings, washings, and biopsies from initially involved areas were negative for tumor. The third complete responder (Patient 13) suffered a fatal myocardial infarction after his fourth chemotherapy dose before bronchoscopy was repeated.

In this study, measurable or evaluable lesions included the chest x-ray in 20 patients, supraclavicular lymph nodes in seven, and liver scan in one. Abnormal bone scans, with or without lytic lesions on bone x-rays, represented the site(s) of metastatic disease in three individuals. Following chemotherapy, maximal clinical response was noted in all sites except bone within 4-6 weeks of the onset of therapy. Measurable tumor shrinkage was often noted within 7-10 days of the onset of treatment. All treatment responses were determined prior to initiation of radiation therapy. Patient survival is indicated in table 2 and figure 1. Eighteen patients have died and two are living, with disease, at 12+ and 19+ months, respectively. The median survival was 6 months, with 30% of patients surviving 1 year.

Treatment Toxicity

The treatment regimen was moderately toxic. Only 14 patients received the planned three cycles (six doses) of remission induction chemotherapy. Five patients received only two cycles of treatment and one received only a single cycle. Poor tolerance for chemotherapy was the basis for shortening the remission induction period. Hematopoietic and renal toxic effects were also noted (table 2). The median nadir wbc count for all induction chemotherapy treatments was 2000/mm^3. Seven patients had nadir wbc counts < 1000/mm^3. In these seven patients, wbc counts remained < 1000/mm^3 for 1-7 days (median, 4). Platelet count nadirs < 100,000/mm^3 occurred in seven patients; platelet count nadirs < 50,000/mm^3 occurred in three patients, all of whom required one or more platelet transfusions. A maximum serum creatinine > 1.5 mg/dl was noted in 12 patients. Serum creatinine returned to normal levels in all patients, except Nos. 19 and 20. Patients 1-18 received repeat courses of cisplatin, at reduced dosage, without incident.

There was minimal alopecia with treatment. No patient developed significant neuropathy and there was no skin

TABLE 2.—Patient characteristics

Patient No.	Age (yrs)	Disease stage	Performance status	Treatment response*	Survival (mos)	Wbc count		Platelet count nadir	Maximum creatinine (mg/dl)
						Nadir	Days < 1000/mm^3		
1	57	$T_3N_2M_0$	2	PR	13	0.2	5	18	1.3
2	64	$T_2N_xM_0$	1	PR	8	0.9	1	92	1.6
3	73	$T_1N_0M_0$	1	CR	19+	0.5	5	26	1.7
4	56	$T_2N_2M_0$	1	PR	16	1.5	—	198	2.2
5	66	$T_2N_2M_0$	1	PR	13	0.9	1	80	1.2
6	70	$T_3N_0M_1$	1	PR	14	2.4	—	220	1.3
7	62	$T_2N_2M_1$	1	PR	4	0.6	3	285	1.4
8	64	$T_3N_2M_0$	1	CR	4	2.1	—	50	1.7
9	58	$T_3N_2M_1$	1	PR	5	2.5	—	230	1.5
10	66	$T_2N_2M_1$	1	PR	5	2.4	—	180	1.9
11	67	$T_2N_2M_0$	1	PR	12+	2.7	—	290	1.3
12	56	$T_3N_2M_0$	1	PR	10	1.8	—	104	2.5
13	58	$T_2N_2M_0$	2	CR	1	4.9	—	335	2.1
14	55	$T_3N_1M_1$	2	PR	4	1.4	—	64	1.6
15	63	$T_3N_2M_1$	2	PR	8	2.4	—	120	2.1
16	67	$T_2N_2M_1$	1	PR	5	7.6	—	190	1.6
17	56	$T_2N_2M_0$	1	PR	3	7.0	—	240	1.2
18	44	$T_2N_2M_0$	2	NR	6	6.6	—	380	0.9
19	65	$T_2N_2M_1$	2	NR	7	0.2	7	10	2.8
20	65	$T_3N_2M_0$	2	NR	2	0.3	7	20	3.0

* PR = partial response; CR = complete response; and NR = no response.

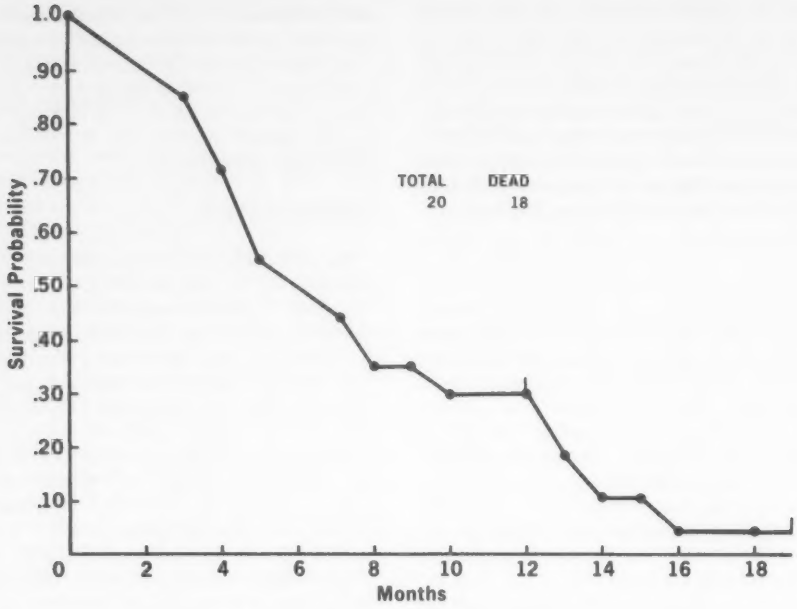

FIGURE 1.—Survival of the 20 study patients.

or lung toxicity attributable to bleomycin administration. Nausea and vomiting were generally controlled with the antiemetics employed.

The RBCD results for each treatment cycle are listed in table 3. The pretreatment RBCD for 19 evaluable squamous cell lung cancer patients was 12.1 ± 4.6 ml of rbc suspension filtered in 10 minutes. This was significantly less than 16.3 ± 4.5 ml/10 minutes recorded for 20 normal controls ($P < 0.01$). As indicated in table 3, there was a tendency for RBCD to decline prior to initiation of each cycle of chemotherapy, although these differences were not statistically significant. Chemotherapy-induced increase in RBCD (≥ 25%) at the time of Treatments 2 and/or 4 occurred in 16 of 17 responding patients and did not occur in any of the three nonresponding patients.

DISCUSSION

The present study is an attempt to test the hypothesis that chemotherapy efficacy can be improved if drugs are administered when RBCD is increased over pretreatment values. As indicated in the introduction, capillary blood flow may be impaired in cancer patients as a result of decreased RBCD. While we do not know what RBCD value, as measured by our method, might be associated with significant impairment of capillary circulation or what RBCD is associated with optimal capillary flow, it was found in this study that responding patients were considerably more likely to increase their RBCD with chemotherapy than were treatment nonresponders.

The favorable results of this study (an overall response rate of 85% with a complete response rate of 15% in 20

TABLE 3.—RBCD changes during treatment

Treatment cycle*	No. of evaluable patients	RBCD (ml/10 mins)	
		Pretreatment	Maximum
1	19	12.1 ± 4.6	13.6 ± 5.6
2	19	11.2 ± 4.7	13.5 ± 5.5
3	11	10.3 ± 5.7	10.2 ± 3.9

* Each treatment cycle consisted of 2 chemotherapy treatments. Treatments 1 and 2 were given during cycle 1, Treatments 3 and 4 during cycle 2, and Treatments 5 and 6 during cycle 3.

patients with advanced squamous cell lung cancer) lend credence to, but do not provide proof of, the above-stated hypothesis. However, all of the drugs used in the present trial have been administered in more conventional schedules in other published lung cancer studies with response rates considerably lower than those achieved in this trial (2,19-21). Survival results in this trial were disappointing, even given the fact that 12 of the 20 patients entered in this study had one or more adverse prognostic features, including performance status of 2, presence of distant metastases, or both. Poor tolerance to chemotherapy, requiring a decrease in the number of remission induction cycles administered to six patients, possibly also contributed to the absence of survival benefit.

The treatment regimen for squamous cell lung cancer reported here, while promising as far as response rates are concerned, obviously requires further improvement. However, this type of chemotherapy approach does open up new avenues of investigation into the treatment of one of the most common solid tumors of man. Trials of lower doses of the same drugs used here, of alternative drugs, and of 2- or 3-day continuous infusions of one or more of the treatment drugs (since chemotherapy enhancement of RBCD generally persists for 2-3 days) are all possible future treatment options to be evaluated.

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Addition of Cisplatin and Bleomycin to Vincristine-Carmustine-Doxorubicin-Prednisone (VBAP) Combination in the Treatment of Relapsing or Resistant Multiple Myeloma: A Southwest Oncology Group Study[1,2]

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Fifty-five patients with myeloma who had relapsed on or were resistant to melphalan and/or cyclophosphamide with prednisone received vincristine, carmustine, doxorubicin, and prednisone (VBAP) plus cisplatin and bleomycin at 21-day intervals. Eighteen (32.7%) patients responded. The response rate was 38.5% (15 responses among 39 patients) in relapsing patients. Three (18.8%) of 16 patients with resistant myeloma responded. Granulocytopenia was the most frequent toxic effect, and was severe in 12 (22%) patients. Severe thrombocytopenia occurred in seven (13%) patients and severe nausea and vomiting occurred in eight (15%). One patient with previously normal renal function developed renal failure on this regimen. The median survival (100 weeks) in those patients responding to treatment was significantly longer than that in patients not responding (25 weeks; $P = 0.001$). VBAP plus cisplatin and bleomycin was at least as effective as VBAP but had greater toxicity, expense, and inconvenience; it therefore is not preferable to VBAP. [Cancer Treat Rep 68:481–485, 1984]

The treatment of multiple myeloma in patients no longer responding to combinations of melphalan and/or cyclophosphamide and prednisone remains a significant management problem. Recently, the Southwest Oncology Group (SWOG) reported encouraging results in this group of patients using a combination of vincristine, carmustine (BCNU), doxorubicin, and prednisone (VBAP) (1). Of note is the fact that none of these agents in combination yielded significant results when used alone.

In an attempt to improve results, agents were sought that might increase the response rate to VBAP without increasing toxicity.

Cisplatin (DDP) had not shown promising results in patients with myeloma (2). However, an increased survival had been demonstrated in mouse myeloma by Ogawa et al (3). Minimal marrow toxicity in doses similar to those used in this study had been reported in phase I trials (4).

Bleomycin (Bleo) had produced a remission in one of 20 patients treated at the M. D. Anderson Hospital (5), but no responses were observed in 14 additional patients (6). However, low-dose Bleo has had a definite added effect in other B-cell malignancies (non-Hodgkin's lymphoma) and has been found to exert a synergistic antitumor effect when combined with DDP in vitro (7).

In a pilot study, several dose regimens were considered. Doses of 20–40 mg/m^2 of DDP for 4 days were added to VBAP. No sign of myelotoxicity was noted at doses of 20–30 mg/m^2 daily for 4 days. The dose-limiting factor in this pilot study was gastrointestinal toxicity. The dose of DDP chosen in the protocol (20 mg/m^2 daily × 4) was a dose that did not produce nausea and vomiting requiring hospitalization.

In summary, the rationale for the addition of DDP and Bleo to VBAP was the low incidence of myelotoxicity using these agents and the studies in other B-cell malignancies that showed synergistic effects using Bleo, doxorubicin, and DDP.

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MATERIALS AND METHODS

A total of 57 patients were entered in the study by 14 SWOG institutions between August 1977 and October 1979. Two patients received no treatment; 55 were eligible for analysis. All patients had progressive symptomatic myeloma.

Thirty-one patients began the study with high tumor mass (56%) using the staging system of Durie and Salmon (8); 13 had intermediate tumor mass (24%); nine had low tumor mass (16%); and two had indeterminate tumor mass.

Thirty-nine (71%) patients started the study with a performance status of at least 50% on the Karnofsky scale.

Thirty-nine (71%) patients had previously responded to chemotherapy. Response had consisted of at least a 75% reduction in the myeloma protein production rate. These relapsing patients demonstrated progressive myeloma protein elevations while receiving the therapy that previously produced response.

Sixteen (29%) patients were considered resistant since they had shown no response to, and had progressive disease while receiving, melphalan and/or cyclophosphamide and prednisone.

Treatment Regimen

When eligible patients had recovered from toxicity from previous chemotherapy, VBAP+DDP+Bleo was begun according to the schema in table 1.

The DDP was infused in a solution of 1 L of 5% dextrose in one-half normal saline with 20 mEq of KCl over a 2–4-hour period. Courses were repeated at 21-day intervals if there was no myelotoxicity. Treatment was delayed until the granulocyte count returned to 1500/mm^3 and the platelet count returned to 100,000/mm^3. BCNU and doxorubicin were decreased to 10 mg/m^2 if the granulocyte count nadir was < 1000/mm^3 or the platelet count nadir was < 100,000/mm^3. BCNU and doxorubicin were decreased to 10 mg/m^2 and the DDP was omitted in the event of increasing BUN and/or creatinine or creatinine clearance that fell to < 40 ml/minute.

Prior to each course, serum calcium, creatinine, uric acid, BUN, creatinine clearance, and ECG were obtained. Anemia and hypercalcemia were treated appropriately during the chemotherapy. As in the prior VBAP study, three courses of therapy were defined as an adequate trial (1).

Response Evaluation

The response criteria were the same as those previously reported in the analysis of the SWOG study using VBAP (1). A decrease of the "M" peak in the serum of ⩾ 50% and a decrease of the "M" peak in the urine if present to < 0.1 g/24 hours on two consecutive determinations were rated a response. No patient was rated responsive if there was progression of other clinical indicators of myeloma.

Confidence interval estimates of response rates were based on the logit transformed sample rates (9), logit (p) = natural log [p/(1-p)], which have approximate variances $[np(1-p)]^{-1}$. Chi-square tests were used to compare response rates. Survival curves were estimated using the method of Kaplan and Meier (10) and were compared using the log-rank test (11).

RESULTS

Responses were documented in 18 of 55 (32.7%) of the eligible patients (95% confidence limits of 30.9, 34.6). The response rate in relapsing patients was 38.5% (15 responses among 39 patients; confidence limits of 35.9, 41.1), and in resistant patients was 18.8% (three responses among 16 patients; confidence limits of 13.3, 25.8). This difference was not statistically significant ($P = 0.27$).

No patient had a response with less than three courses of VBAP+DDP+Bleo. The response rate in the 40 patients receiving an adequate trial was 45% overall and was 48.4% and 33.3% for patients with relapsing and resistant myeloma, respectively.

TABLE 1.—Treatment schema of VBAP+DDP+Bleo

Drug (dose unit), route	Drug dose on Day—				
	1	2	3	4	5
Vincristine (total mg), iv	1				
BCNU (mg/m^2), iv	30				
Doxorubicin (mg/m^2), iv	30				
Prednisone (mg/m^2), orally	60	60	60	60	60
Bleo (total mg), im		7.5	7.5	7.5	7.5
DDP (total mg), iv		20	20	20	20

TABLE 2.—Treatment of relapsing or resistant myeloma: immunoglobulin class

	VBAP		VBAP+DDP+Bleo	
	% of patient total No.	% response	% of patient total No.	% response
IgG	42	26	40	41
IgA	25	14	15	25
Bence Jones	21	38	7	0
IgG + Bence Jones	10	27	27	33
IgA + Bence Jones	0	—	9	40
Serum peak only	69	22	55	35
Bence Jones present	31	34	43	29

Fifteen (37.5%) patients did not receive an adequate trial of VBAP+DDP+Bleo. Nine patients died; two patients refused further therapy with this combination, while in an additional two patients therapy was discontinued due to toxicity; one patient's therapy was discontinued when he had a stroke; and one patient was lost to follow-up.

The relationship between response rate and myeloma protein type is shown in table 2.

Toxicity

Some form of toxicity was observed in 50 of the 55 eligible patients (table 3). Granulocytopenia was the most common toxic effect, with 35 (64%) patients experiencing at least moderate toxicity. Granulocyte transfusion support was required in three (5%) patients. Thrombocytopenia was severe in seven patients (13%) and required platelet transfusions in two (4%). Nausea and vomiting of significant degree was reported in 13 (24%) patients and was life-threatening in one. This patient was removed from protocol after just one course of treatment. No nausea or vomiting was recorded in more than one-half of the patients. One patient with IgG myeloma experienced severe renal failure requiring dialysis. This patient had not had previous renal disease or proteinuria. Twenty-six (47%) patients were reported to have severe toxic effects of various forms. Treatment was not altered in the patients with alopecia or hearing loss. Treatment had to be significantly altered, delayed, or discontinued in 23 (42%) patients. Conversely, VBAP+DDP+Bleo was well-tolerated in 29 (53%) patients.

Survival

Survival from the start of treatment was determined in all 55 patients. Thirty-eight patients had died by the time of analysis. The overall median survival was 33 weeks. Survival was virtually identical for resistant and relapsing patients, with medians of 33 and 36 weeks, respectively ($P = 0.94$).

Objective response to VBAP+DDP+Bleo was strongly related to survival. In the adequately treated group the median survival in responding patients was 100 weeks

TABLE 3.—VBAP+DDP+Bleo in 55 patients with relapsing or resistant myeloma: type and degree of toxicity*

Toxic effect	Grade—				
	0†	1	2	3	4
Granulocytopenia‡	14	6	23	9	3
Thrombocytopenia§	33	6	9	5	2
Anemia	52	1	2		
Peripheral neuropathy	54		1		
Diarrhea	54		1		
Nausea/vomiting	29	13	5	7	1
Alopecia	52		1	2	
Paresthesia	54		1		
Renal	54			1	
Chills/fever	54		1		
Pulmonary	54			1	
Hearing loss	54			1	

* Values in table = No. of patients.

† None or not reported.

‡ Granulocyte count nadirs (cells/mm^3): grade 1—2000-2499; grade 2—1000-1499; grade 3—500-999; and grade 4—< 500.

§ Platelet count nadirs (cells/mm^3): grade 1—75,000-99,999; grade 2—50,000-74,999; grade 3—25,000-49,999; and grade 4—< 25,000.

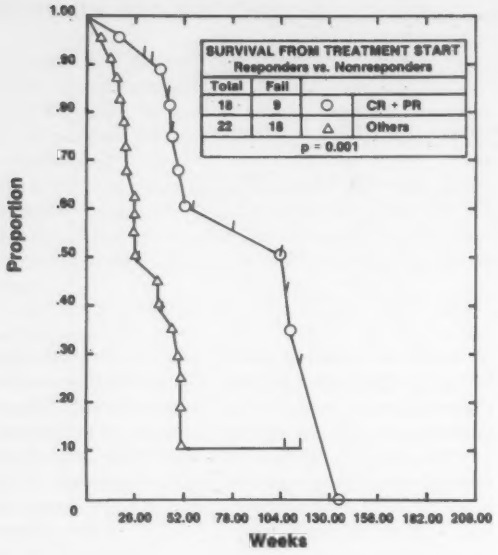

FIGURE 1.—SWOG-7719: survival among patients with relapsing or resistant myeloma treated with VBAP+DDP+Bleo.

and for nonresponding patients was 25 weeks. The survival curves were significantly different ($P = 0.001$) (fig 1). This difference is not diminished when comparisons are adjusted for either performance status at start of treatment or pretreatment tumor mass category.

DISCUSSION

Using VBAP in patients with myeloma relapsing on melphalan and/or cyclophosphamide and prednisone, the SWOG reported a 30% response rate. VBAP produced two responses among 28 (7%) patients who had never responded (resistant). The median survival was increased significantly in responding patients.

DDP and Bleo were added to VBAP with the intent to increase the response and survival without increasing toxicity in both relapsing and resistant patients with myeloma. A comparison of results from the current VBAP+DDP+Bleo study with those from the VBAP study is summarized in tables 2, 4, and 5. The response rates and survival in the various categories examined were not statistically significant. In previous responders to alkylating agents and in patients who can be treated adequately there appears to be a good response rate when one considers that the patients had failed treatment at the time they were given either VBAP regimen.

The patient populations in the two studies differed in two major categories: tumor mass and immunoglobulin class. All patients in the VBAP study were in the high tumor mass category, while 44% of the patients had lower tumor mass in the present study. The immunoglobulin class and response are shown in table 2. The occurrence and response of myeloma patients with Bence Jones protein only was quite different between the two studies. Three times as many patients in this study had myeloma protein peaks in both urine and serum, compared with the study of VBAP alone. All immunoglobulin categories except Bence Jones only responded better to VBAP+DDP+Bleo. In late-stage disease the influence of tumor mass and immunoglobulin class of myeloma protein on response and survival is unknown. Therefore, comparison of results in these studies is tenuous. Future studies in patients with relapsing or resistant myeloma should include analysis of extent of disease and immunoglobulin class to determine whether these factors do influence response and survival in late-stage patients.

Toxicity was more severe when DDP and Bleo were added to VBAP. Nausea and vomiting required hospitalization in eight (15%) patients. Treatment was discontinued in one patient due to gastrointestinal side effects. The incidence of serious thrombocytopenia was equal in both combinations. Serious granulocytopenia was significantly greater with VBAP+DDP+Bleo. One patient with myeloma who had normal renal function at the start of treatment developed renal failure while receiving VBAP+DDP+Bleo. This observation warrants comment, since it underscores the potential nephrotoxic effects of even low-dose DDP in patients who may have subclinical renal disease.

The doses of DDP and Bleo were quite low. There is the

TABLE 4.—Treatment of relapsing or resistant myeloma: results of VBAP with or without DDP+Bleo

	VBAP	VBAP+DDP+Bleo
Response (No. of patients responding/ No. treated (%)		
Overall	39/151 (25)	18/55 (33)
Relapsing patients	37/123 (30)	15/39 (99)
Resistant patients	2/28 (7)	3/16 (19)
Adequately treated patients	39/122 (32)	18/40 (45)
Median survival in wks		
Overall	33	33
Responding patients	78	100
Nonresponding patients	33	25

TABLE 5.—VBAP toxicity with or without DDP + Bleo: patients experiencing severe toxicity

Toxic effect	No. of patients (%)	
	VBAP (total: 151 patients)	VBAP + DDP + Bleo (total: 55 patients)
Granulocytopenia	2 (1)	7 (13)
Thrombocytopenia	10 (7)	3 (5)
Gastrointestinal	2 (1)	8 (15)
Overall	14 (9)	23 (42)

possibility that the response could be improved by increasing the amounts of either or both agents. However, it does not appear prudent to attempt this in future trials, in view of the increased side effects patients experienced in this schedule.

VBAP may be given in 1 day. VBAP + DDP + Bleo in this study was administered over a 5-day period every 21 days. This schedule causes an increased expense and inconvenience to the patients.

The addition of DDP and Bleo to VBAP did show a slight though statistically insignificant increase in response in patients with relapsing or resistant myeloma. However, considering the increased toxicity, expense, and inconvenience, this schedule of VBAP + DDP + Bleo offers no advantage over VBAP alone in late-stage myeloma failing melphalan and/or cyclophosphamide and prednisone therapy. We believe that VBAP remains the preferred treatment for these patients.

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Doxorubicin (75 mg/m^2) for Hepatocellular Carcinoma: Clinical and Pharmacokinetic Results[1,2]

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Of 157 consecutive patients who had histologically diagnosed hepatocellular carcinoma, 52 with a good performance score, bilirubin < 2 mg/dl, and absence of bloody ascites were treated with a 75-mg/m^2 dose of doxorubicin (unadjusted for hepatic function) every 3 weeks in a prospective trial. Forty-six patients were treated in West Africa and six in Southern California. No complete responses were seen and only six patients (11%) achieved partial objective responses. Plasma concentrations of doxorubicin and doxorubicinol (adriamycinol) were determined at four selected time points for up to 72 hours, corresponding to the terminal phase of disposition in eight African patients. The African patient results were compared to those seen in North American patients with hepatocellular carcinoma and other malignancies. In African patients with hepatocellular carcinoma, terminal half-life of doxorubicin was 31.1 ± 4.7 hours (mean ± SD), while the apparent half-life of doxorubicinol was prolonged at 39.8 ± 15.9 hours. The ratios of the corresponding concentration × time values of doxorubicinol to doxorubicin, which reflect the overall metabolite to parent drug ratio, ranged from 0.7 to 4.6, with a mean ratio of 2.03 ± 1.20 in the African patients with hepatocellular carcinoma compared to a mean ratio of 0.76 ± 0.31 in North American patients with other malignancies. Pharmacokinetic findings in hepatocellular carcinoma patients in North America and Africa were similar, reflecting elevation and prolongation of doxorubicinol metabolite relative to doxorubicin in the plasma of patients with this disease from both areas. We conclude that: (*a*) doxorubicinol disposition is altered in African as well as North American patients with hepatocellular carcinoma; and (*b*) even when given in full dose to patients with favorable prognostic features, iv doxorubicin has only limited activity against hepatocellular carcinoma. [Cancer Treat Rep 68:487–491, 1984]

Unresectable hepatocellular carcinoma is one of the most rapidly fatal tumors in man (1,2). Results with chemotherapy were disappointing until treatment with a 75-mg/m^2 doxorubicin dose was reported to achieve a high response frequency in African patients with this disease (3). Although other investigators have confirmed the activity of doxorubicin against hepatocellular carcinoma, the high response frequency of the original report has not been duplicated in subsequent studies, all of which used a lower dosage of doxorubicin adjusted for hepatic dysfunction (4–9). Recently, we have observed (9,10) that in North American patients with hepatocellular carcinoma, even moderate hepatic dysfunction did not significantly alter doxorubicin disposition except to delay the formation and elimination of doxorubicinol (adriamycinol). This pharmacokinetic result combined with the clinical observation of unusual tolerance to doxorubicin seen in several hepatocellular carcinoma trials (3,4,8,9) suggested a possible explanation for the lack of therapeutic response seen when doxorubicin dosage was attenuated for hepatic dysfunction in patients with primary liver cancer. To evaluate the hypothesis that hepatocellular carcinoma patients could benefit from higher dosages of doxorubicin than those previously given, we prospectively studied the use of 75-mg/m^2 doxorubicin therapy, unadjusted for hepatic dysfunction, in patients with good

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performance status in Africa and North America. In addition, the influence of hepatocellular carcinoma on the pharmacokinetic disposition of doxorubicin was investigated in several African patients in this trial and compared to results previously obtained in North American patients with this disease.

MATERIALS AND METHODS

All patients had histologically confirmed, surgically unresectable hepatocellular carcinoma. Favorable prognostic features which were required for entry included: (*a*) bilirubin < 2 mg/dl; (*b*) Karnofsky performance score ⩾ 70%; (*c*) absence of bloody ascites; and (*d*) no prior surgical resection, chemotherapy, or radiotherapy. A measurable disease parameter was required. Contraindications for patient eligibility included a granulocyte count < 1700/mm^3 and/or a platelet count < 125,000/mm^3. Patients with hypersplenism but decreased platelet count and normal platelet generation in bone marrow were eligible for entry. Excluded from the study were patients with symptomatic congestive heart failure, acute myocardial infarction within the previous 2 months, and renal disease with creatinine > 2.5 mg/dl. Pretreatment study observations, made within 7 days of starting treatment, included complete history and physical examination, ECG, liver technetium scan, serum alpha-fetoprotein determination, hepatitis B surface antigen, chest radiograph, and liver function tests.

All patients were treated with doxorubicin given by injection at a dose of 75 mg/m^2. Treatment was repeated at 3-week intervals if hematologic parameters continued to meet entry criteria and if disease progression did not occur. Repeat assessment of measurable disease sites was performed at monthly intervals and liver scans were repeated every 2 months. Criteria of response were as follows: (*a*) *complete response* was defined as the disappearance of all tumor, with subjective improvement lasting at least 1 month; (*b*) *partial response* was defined as a > 50% reduction in the sum of the perpendicular diameters of all measurable lesions, without evidence of new lesions, associated with subjective improvement lasting at least 1 month; and (*c*) if hepatomegaly was the measurable parameter for partial response, reduction of the sum of liver measurements below each costal margin at the midclavicular lines and from the tip of the xiphoid cartilage by at least 30% was required. Patients were considered evaluable for toxicity and response after receiving one dose of doxorubicin.

From several previous studies including those in our laboratory (11,12), the plasma disposition profiles of doxorubicin in patients have been found to be triphasic, with the terminal phase for most patients beginning about 6 hours after administration. Since the postdistributive phase for most drugs is more instructive in reflecting the elimination of a drug than the distributive phase, plasma samples were drawn from hepatocellular carcinoma patients at 0, 8, 24, 48, and 72 hours for pharmacokinetic analysis of doxorubicin disposition, with slight variations for individual patients. These time points were found to be adequate for monitoring the elimination phase of doxorubicin and the detection of a possible doxorubicinol peak in plasma. The plasma samples were kept frozen at -20°C and were transported to the laboratory in the United States, where analysis was performed within 3 months of drug administration; < 10% degradation of the drug was found when separate plasma samples containing doxorubicin and doxorubicinol were stored under similar conditions. The concentrations of doxorubicin and doxorubicinol in the samples were analyzed by a thin-film fluorescence scanning method (13).

Patients with hepatocellular carcinoma were entered from two areas: Southern California in the United States and Fajara, Gambia, in West Africa. All patients treated in the Gambia were admitted to the British Medical Council Research Hospital in Fajara and remained hospitalized and under observation during their entire clinical course. Histologic confirmation of diagnosis in African patients was obtained using aspiration cytology of the liver. All cytologic evaluation was done by Dr. Elizabeth Hudson, cytopathologist at the Northwick Park Hospital in Harrow, Middlesex, England. Patients from the United States were examined at the Harbor-UCLA Medical Center, Torrance, CA, and the Huntington Memorial Hospital, Pasadena, CA. Histologic confirmation of diagnosis in American patients was obtained using hepatic biopsy specimens read at the University of Southern California School of Medicine, Department of Pathology.

RESULTS

Over the 18-month period of study a total of 157 consecutive patients with a diagnosis of hepatocellular carcinoma were seen in both locations (143 patients in Africa and 14 patients in North America). Of these 157 patients, 52 met entry criteria (46 in Africa and six in North America) and were treated with doxorubicin in this trial. Almost all patients not meeting entry criteria had poor Karnofsky performance scores or bilirubin levels > 2 mg/dl.

All 52 patients entered in the study received at least one dose of doxorubicin and were therefore considered evaluable for toxicity and response. Clinical characteristics of these patients included a median age of 49 years (range, 28-65), a median Karnofsky performance score of 76%, and the presence of metastatic disease to bone or lung in 14%. Alpha-fetoprotein was initially elevated (> 100 ng/ml) in 72% of the patients. In 56% of the patients, hepatitis B surface antigen was present. Pretreatment transaminases (SGOT or SGPT) were elevated in 98% of patients; bilirubin elevation (> 1.2 but < 2.0

TABLE 1.—Response and toxic effects of 75 mg/m^2 of doxorubicin in patients with hepatocellular carcinoma

	No. of patients (%)
Total with hepatocellular carcinoma evaluated	157 (100)
Eligible for entry in the study*	52 (33)
Response	
Complete response	0 (0)
Partial objective response	6 (11)
Toxic effects	
Leukocyte count nadir (cells/mm^3)	
1500–3000	16 (31)
< 1500	9 (18)
Platelet count nadir (cells/mm^3)	
< 75,000	
Gastrointestinal	5 (9)
Nausea/vomiting	
Mild/moderate	24 (46)
Severe	3 (5)
Stomatitis	3 (5)

* All 52 entered patients were evaluable for toxicity and response.

mg/dl) was found in ten patients prior to treatment. All 46 African patients were black as was one of six patients from North America. Except for this racial difference, the pretreatment characteristics of patients from North America and Africa were similar.

Information on the response to doxorubicin therapy is summarized in table 1. No complete responses to doxorubicin were observed. Partial responses were seen in only six of 52 patients (11%); two patients had decreased hepatomegaly and four had a > 50% reduction in filling defect size on liver scan as their response parameter. Responses were seen in both African (five responses among 46 patients) and North American (one response among six patients) patients. All responses were seen in patients with alpha-fetoprotein elevation. In five of the six responding patients, objective decrease in tumor size was associated with reduction in alpha-fetoprotein level. Despite pre-entry selection of patients with favorable prognostic features, only 25 of the 52 patients (45%) received more than one course of doxorubicin treatment; therapy was stopped in all cases because of disease progression or death. A total of 138 courses of doxorubicin were given to the 52 patients; patients received a median of 2.1 courses. Median survival was 4.2 months, with no patient surviving > 1 year.

The toxic effects of doxorubicin therapy are shown in table 1. Appreciable hematologic toxicity occurred with this regimen, with 49% of patients having a wbc count of < 3000/mm^3. No patient developed clinical congestive heart failure or experienced local problems related to doxorubicin extravasation.

Plasma profiles of doxorubicin and doxorubicinol were obtained for eight African patients. In most patients, the plasma time course of doxorubicin and doxorubicinol showed essentially log-linear declines as expected. The terminal half-life of doxorubicin and doxorubicinol was estimated either from linear regression (when the sample number was 4) or by manual estimation (when the sample number was < 4) of the log concentration versus time plots. In one patient, the doxorubicin and doxorubicinol levels were both significantly prolonged so that accurate assessment of the half-life was not possible. The regression coefficients for doxorubicin disposition in those patients which could be analyzed by the linear regression method ranged from 0.7 to > 0.9. Similarly, the apparent terminal half-life of doxorubicinol in these patients was also estimated. These values, along with liver functions at the time of study, are illustrated in table 2. As seen, disposition of doxorubicin and doxorubicinol in the African patients with hepatocellular carcinoma was not apparently influenced by elevation in transaminase. The average terminal half-life for doxorubicin and the apparent half-life for doxorubicinol were found to be 31.1 ± 4.7 and 39.8 ± 15.9 hours, respectively. The corresponding areas under the concentration × time curve (C × T) during the sampling period (8–72 hours) for doxorubicinol and doxorubicin were also estimated by the trape-

TABLE 2.—Doxorubicin disposition in African patients with hepatocellular carcinoma

Patient No.	Liver function (IU)*		Doxorubicin		Doxorubicinol	
	SGOT	SGPT	Half-life (hrs)	C × T† (ng/ml/hr)	Half-life (hrs)	C × T‡ (ng/ml/hr)
1	57	130	32	2022	67	5559
2	14	324	36	1084	41	1621
3	26	125	30	1602	30	2908
4	21	77	33	1884	33	1310
5	25	135	25	1399	25	1863
6	40	275	35	1713	55	3677
7	80	171	Prolonged	650	Prolonged	2985
8	17	58	27	776	27	1072
Mean ± SE			31.1 ± 4.7		39.8 ± 15.9	

* < 40 IU, within normal limits.
† Calculated from 8- to 72-hr interval.
‡ Calculated from absolute doxorubicinol levels, which are obtained by multiplying the doxorubicin equivalent values by a factor of 1.5 (ref 10).

TABLE 3.—C × T ratios (doxorubicinol/doxorubicin) in American and African patients with hepatocellular carcinoma compared to patients with other malignancies

Patient group		No. of patients evaluated	Doxorubicinol C × T/ doxorubicin C × T*
Area	Disease		
Africa	Hepatocellular carcinoma†	8	2.03 ± 1.20
North America	Hepatocellular carcinoma	8	2.36 ± 2.12
North America	Other primary sites of malignancy†	7	0.76 ± 0.31

*Mean ± SD.
†From previously published results (9).

zoidal method and expressed as nanograms per milliliter per hour. The ratio of doxorubicinol to doxorubicin, reflecting their average relative levels, was also calculated and is compared to values previously obtained (9,10) in North American patients with hepatocellular carcinoma and those with other primary cancer sites in table 3.

DISCUSSION

Previous trials with doxorubicin have suggested a possible dose-response relationship between doxorubicin and hepatocellular carcinoma, since patients receiving higher drug dosage experienced higher response frequency (5,7). In those studies, prognostic factors and doxorubicin dosage levels could not be separated, since "good-risk" patients with relatively normal hepatic function received higher doxorubicin dosage than "poor-risk" patients with abnormal liver function. In the current trial, despite the successful administration of a 75-mg/m^2 doxorubicin dosage to a large number of good-performance patients with hepatocellular carcinoma, only limited clinical antitumor effectiveness was seen. Clinical tolerance was acceptable, with about one-half of the patients experiencing nausea or vomiting, but objective evidence for response was seen in only 11% of the 52 patients treated. This result, coupled with those from other trials using strict criteria of response, suggests only limited effectiveness for iv doxorubicin alone (4–6) or in combination (6,9,14) in the treatment of hepatocellular carcinoma in both Africa and North America.

Patient selection is a well-recognized factor which may influence clinical trial results, especially in pilot studies involving small numbers of patients. Most often, it is unclear what criteria have been used to select patients for study. In the current report, all patients seen with a diagnosis of hepatocellular carcinoma over an 18-month period at the participating institutions were evaluated; only one-third (52 of 157 patients) met the entry criteria of low bilirubin level, absence of bloody ascites, and good performance status. In prior trials, most patients with hepatocellular carcinoma responding to doxorubicin did not have markedly elevated bilirubin or decreased performance scores (5,7,9). As a result, even the 11% response frequency with doxorubicin seen in our patients might not be achieved when a population with less favorable prognostic features is treated. Although several studies (15,16) have suggested benefit for use of iv doxorubicin in selected hepatocellular carcinoma patients, most larger series support a cumulative response frequency of < 20% (17). Since this level of response to doxorubicin therapy does not exceed that obtained with other therapeutic approaches, doxorubicin does not constitute "standard therapy" for hepatocellular carcinoma, and patients with this disease should be considered candidates for investigative protocols.

The average half-life of doxorubicin in the eight African patients in this trial was 31.1 ± 4.7 hours, a value which is similar to that reported previously for patients with hepatocellular carcinoma and abnormal liver function in North America (9,10) and is within the range observed for patients with other diagnoses and normal liver function (9,11,18). On the other hand, the apparent terminal half-life of doxorubicinol in the African hepatocellular carcinoma patients was prolonged, ranging from 25 to 67 hours with an average value of 40 hours. Although the true half-life of doxorubicinol in patients with normal hepatic function has not been determined, it is believed to be similar to that of doxorubicin, since direct administration of doxorubicinol into rabbits has resulted in a plasma half-life of approximately 30 hours, essentially the same as that of doxorubicin (19). The ratio of doxorubicinol to doxorubicin, which reflects their average relative levels and changes in clearances (20), ranged from 0.7 to 4.6, with a mean of 2.03 ± 1.02 in the African hepatocellular carcinoma patients compared to a mean ratio of 0.76 ± 0.31 found in North American patients with other malignancies. This comparison involves patients receiving different doxorubicin doses, with the North American patients receiving lower doses. However, in rabbits the doxorubicinol/doxorubicin ratio decreased as the doxorubicin dose increased (21). Thus, since the elevation of the doxorubicinol/doxorubicin ratio seen in hepatocellular carcinoma patients is a reversal of the expected trend, it may well represent altered doxo-

rubicinol disposition associated with primary liver cancer. This altered drug disposition in patients with hepatocellular carcinoma given doxorubicin can perhaps be explained by the presence of malignant hepatocytes which retain some variable amount of the usual metabolic capacity of normal hepatocytes. In any event, the clinical significance of the delayed doxorubicinol disposition in these patients will require further study, since doxorubicinol is cytotoxic to malignant cells in some in vitro systems (18).

CONCLUSIONS

An increase in and prolongation of doxorubicinol levels in plasma relative to those of doxorubicin occur in patients with hepatocellular carcinoma. Despite good clinical tolerance, doxorubicin given iv at a 75-mg/m^2 dose to patients with good performance status has only limited activity in hepatocellular carcinoma.

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Comparison of Combination Chemotherapy Programs in Advanced Adenocarcinoma-Large Cell Carcinoma of the Lung: A North Central Cancer Treatment Group Study[1,2]

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One hundred and six patients with advanced adenocarcinoma and large cell carcinoma of the lung with no prior chemotherapy were entered in a prospectively randomized trial comparing cyclophosphamide, doxorubicin, and cisplatin versus methotrexate, doxorubicin, cyclophosphamide, and lomustine. The two regimens resulted in nearly identical regression probabilities (36% vs 34%), distributions of time to progression, and survival distributions. The major toxic effects from both regimens consisted primarily of myelosuppression and nausea and vomiting, with severe vomiting occurring more frequently in patients treated with cyclophosphamide, doxorubicin, and cisplatin (43% vs 19%). [Cancer Treat Rep 68:493–498, 1984]

The combination of cyclophosphamide (CTX), doxorubicin (ADR), and cisplatin (CCDP), known as CAP, has been reported to have antitumor activity against large cell (LGC) carcinoma and adenocarcinoma (ACA) of the lung by Eagan et al (1). In their series of patients with and without prior chemotherapy, objective tumor regressions were noted in eight of 22 patients (36%) with ACA and in six of 16 (38%) with LGC carcinoma. Hematologic toxicity from the CAP regimen was generally mild. Unfortunately, severe nausea and vomiting were common among those patients treated with CAP.

Chahinian et al (2) reported on the combination of methotrexate (MTX), ADR, CTX, and lomustine (CCNU), known as MACC, in 83 patients with bronchogenic carcinoma. Only three patients had had prior chemotherapy. Fifteen of 26 patients (58%) with ACA and seven of 20 (35%) with LGC carcinoma had objective tumor regressions, for an overall regression probability of 48% in these cell types. The major symptomatic toxic effects were nausea, vomiting, and stomatitis. Leukopenia was generally mild, and thrombocytopenia was relatively infrequent.

The study reported here was conducted for two reasons: first, to confirm the reported antitumor activity of the CAP and MACC regimens in patients treated in the community clinics of the North Central Cancer Treatment Group; and second, to compare the CAP and MACC regimens in patients with metastatic, recurrent, or unresectable LGC carcinoma and ACA of the lung with respect to regression probabilities, times to disease progression, survival, and toxicity.

STUDY DESIGN AND TREATMENTS

A prospectively randomized study was conducted by the North Central Cancer Treatment Group. To be eligible, patients were required to have histologic or cytologic proof of metastatic, recurrent, or unresectable LGC carcinoma or ACA of the lung. Contraindications to entry included wbc or platelet counts below the lower limits of institutional normal levels, any elevation of direct bilirubin or serum creatinine above the upper limits of institutional normal levels, Eastern Cooperative Oncology Group (ECOG) performance score of 4, any active cardiac dis-

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ease, prior chemotherapy, active uncontrolled infection, or prior major surgery or radiation within 4 weeks. An informed written consent was obtained from all patients prior to entry in the study. After stratification, patients were randomized to receive either CAP or MACC. Patients who progressed on these regimens and who met certain eligibility criteria to receive further chemotherapy were randomly assigned to receive either vincristine or vinblastine.

To assess regression probability and progression of disease, standard definitions of regression, stability, and progression were used (3). These definitions depend on whether the patient had measurable or evaluable (ie, assessable but not measurable) disease. Tumor regressions included complete and partial regressions in measurable disease and complete regression and regression in evaluable disease.

The doses of CTX (400 mg/m^2 iv) and ADR (40 mg/m^2 iv) were identical in the two regimens. The CAP group received 40 mg/m^2 iv of CDDP, while the MACC group received 40 mg/m^2 iv of MTX and 30 mg/m^2 orally of CCNU. All iv administered drugs were rapidly infused over 5-15 minutes without special hydration or diuretics. All treatments were given on Day 1 and were scheduled to be repeated every 4 weeks from Day 1. The maximum permissible total cumulative dose of ADR was 450 mg/m^2. Weekly wbc and platelet counts were required between treatments.

Within the first year following randomization, radiation therapy was allowed in standard palliative dose schedules to nonindicator lesions on one occasion; after 1 year of chemotherapy, radiation was allowed to any and all lesions at the investigator's direction.

The doses of CTX, ADR, and CCNU were modified in subsequent cycles to obtain wbc count nadirs of between 2000 and 3000/mm^3 or platelet count nadirs of between 75,000 and 100,000/mm^3. ADR and MTX were reduced if moderate to severe stomatitis and/or diarrhea were observed. Doses of CDDP and MTX were modified based on the serum creatinine level at the time of re-treatment.

Patients

The study was designed for 100 patients in each regimen, to obtain adequate sensitivity to detect a 50% improvement in median survival of CAP over MACC. Accrual was terminated after 112 patients had been randomized, due to the results obtained at an interim analysis. Of the 112 patients, six were found to be ineligible. One patient randomized to CAP died prior to beginning treatment. Four patients receiving MACC had no measurable or evaluable disease, while a fifth patient had significant cardiac disease at randomization. Of the 106 eligible patients, 53 were randomized into each of the two regimens under study.

Within each institution, patients were stratified according to ECOG performance scores (0-1 vs 2-3), cell type (ACA vs LGC carcinoma), and disease status (measurable vs evaluable). The distribution of each of the stratification covariates and other pretreatment characteristics by treatment is shown in table 1 for the 106 eligible patients. The two regimens were well-balanced with respect to these covariates. As dictated by design, each participating institution contributed nearly one-half of its patient entries to each regimen.

RESULTS

Regression Probability and Time to Progression

Objective tumor regressions were noted in 19 of 53 patients (36%) treated with CAP and in 18 of 53 (34%) treated with MACC. Thirty-five percent of the patients with ACA receiving CAP experienced regressions compared to 24% receiving MACC, while 37% of the patients with LGC carcinoma receiving CAP regressed compared to 50% receiving MACC (table 2). This apparent advantage of CAP in ACA and MACC in LGC carcinoma may well be due to factors other than treatment itself. Specifically, in patients with LGC carcinoma, the MACC regimen had a higher percentage than the CAP regimen of

TABLE 1.—Pretreatment characteristics of the 106 eligible patients*

Characteristic	Treatment regimens	
	CAP	MACC
Eligible patients	53	53
Median age in yrs (range)	61 (37-80)	59 (31-78)
Sex (male:female)	37:16	36:17
ECOG performance score (0-1:2-3)	36:17	39:14
Cell type (ACA:LGC carcinoma)	37:16	33:20
Indicator disease status (measurable:evaluable)	31:22	33:20
Prior resection (yes:no)	10:43	7:46
Prior radiation therapy (yes:no)	6:47	9:44
Extent of disease (limited: metastatic:recurrent)	12:38:3	10:40:3

* Unless specified otherwise, values = No. of patients.

TABLE 2.—Treatment and tumor regression

Characteristic	Treatment regimen*	
	CAP	MACC
All patients	19/53 (36)	18/53 (34)
LGC carcinoma only	6/16 (37)	10/20 (50)
ACA only	13/37 (35)	8/33 (24)
Limited disease only	6/12 (50)	4/11 (36)
Metastatic or recurrent disease only	13/41 (32)	14/42 (33)
Measurable disease	9/31 (29)	13/33 (39)
Evaluable disease	10/22 (45)	5/20 (25)
ECOG performance score		
0-1	15/36 (42)	13/39 (33)
2-3	4/17 (24)	5/14 (36)

* Values = No. of patients with tumor regression/No. of patients treated (%).

patients who were female, had low performance score, or had limited disease. These are all prognostically favorable criteria. Conversely, in patients with ACA, CAP was the regimen with the higher percentage of these types of patients.

Of those patients experiencing regression of disease, seven (13.2%) had complete regression of tumor while receiving CAP, as did four (7.5%) receiving MACC. The median interval from entry to disease progression for responding patients was 9 and 7 months on CAP and MACC, respectively.

Our estimates of time to onset of tumor progression are nearly identical following initiation of either the CAP regimen or the MACC regimen (fig 1). All curves are generated via the Kaplan-Meier technique (4). The median time to tumor progression was 2.9 months for CAP versus 3.9 months for MACC. Only one patient remained on study free of progression in excess of approximately 1 year, with that patient still on study receiving MACC nearly 3 ½ years after initiation of that therapy.

Gehan-Wilcoxon (5) and log-rank (6) two-sided test procedures indicated that the estimated curves in figure 1 are entirely consistent with the hypothesis of equality of time to progression distributions following CAP and MACC, yielding P values of 0.61 and 0.73, respectively. The measure of the association between treatment and time to progression was unaffected when adjustment was made for each of the covariates in table 1 via the Cox regression model (7). On the other hand, the Cox model indicated that the curves were inconsistent with a 50% improvement of CAP over MACC in time to progression (ie, a hazard ratio of 1.5). The one-sided P value, unadjusted for repeated testing, was 0.014.

Survival

The survival following randomization to the CAP and MACC regimens for the 106 eligible patients is presented in figure 2. The curves are remarkably similar, with medians of 7.9 months for CAP and 7.5 months for MACC. The only living patient in this analysis is a 48-year-old female with metastatic LGC carcinoma who has continued in regression for nearly 3 ½ years.

It is obvious that the curves are entirely consistent with the hypothesis of no difference in true survival following CAP and MACC treatments, with Gehan-Wilcoxon and log-rank two-sided P values of 0.94 and 0.88, respectively. However, analysis using the Cox model has revealed that the estimated survival curves are inconsistent with the hypothesis that CAP will provide 50% longer survival than MACC. The one-sided P value of 0.013 from the test of this hypothesis, together with the relatively increased occurrence of nausea and vomiting following CAP without an apparent increase in regression rates or time to progression, led to the early termination of this trial for which an accrual of 200 patients had been planned. Some upward adjustment of this small P value is necessary, due to the effect of several interim analyses performed in this study (8).

Inclusion of covariates in our survival analysis led to the confirmation of the frequently observed result (9-11) that performance status is the strongest predictor of patient survival.

Toxic Effects

The major subjective toxic effects from both regimens consisted primarily of nausea and vomiting. Ninety-four percent of the patients receiving CAP and 77% of the patients receiving MACC experienced vomiting; it was severe in 23 of 53 (43%) patients receiving CAP, compared to only ten of 53 (19%) patients receiving MACC (two-sided Pearson chi-square, $P = 0.006$). Diarrhea was observed more frequently on the CAP regimen (15% vs 9%), while stomatitis was observed more commonly on the MACC regimen (30% vs 13%).

Table 3 indicates that the two regimens produced similar myelosuppression, with the MACC regimen yielding somewhat more frequent thrombocytopenia. A possible drug-related death occurred after a patient's wbc

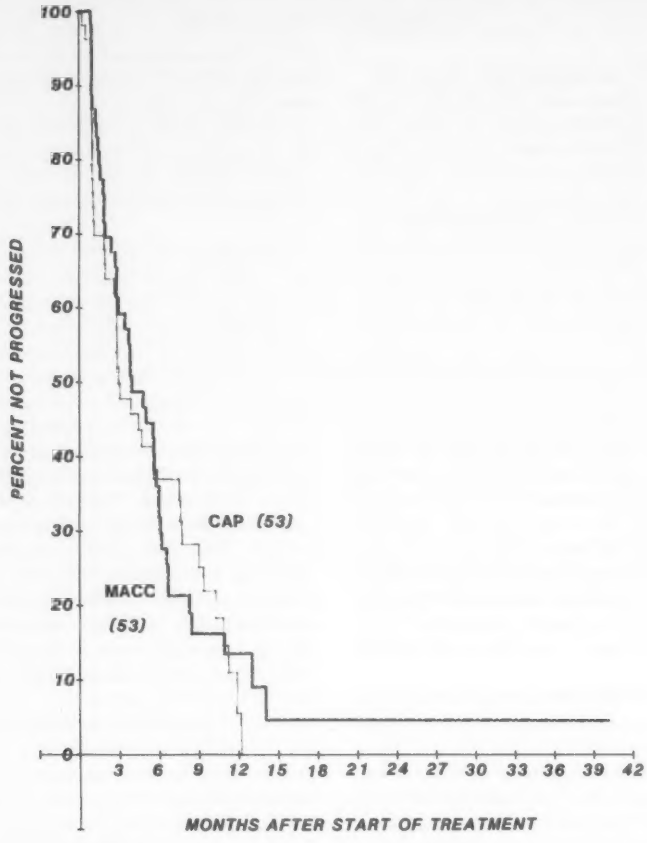

FIGURE 1.—% of patients free of tumor progression following initiation of the MACC and CAP regimens.

count fell to 350/mm^3 following three courses of treatment on the MACC regimen. Renal toxicity was tolerable, with only one patient (receiving CAP) developing a serum creatinine > 1.7 mg/100 ml.

DISCUSSION

This study was designed to address two major goals. The first goal was to determine whether we could confirm, in the patient population identified by the clinics of the North Central Cancer Treatment Group, the reported antitumor activity of the CAP and MACC regimens.

With the CAP regimen as delivered in this study, Eagan et al (1) obtained regression probabilities in previously untreated patients of 43% (six of 14 patients) in ACA and 22% (two of nine) in LGC carcinoma. Subsequently, Knost et al (12) noted regressions in 24% (four of 17) of patients with ACA and in 38% (six of 16) of patients with LGC carcinoma, while Evans et al (13) reported regressions in 28% (14 of 50) of patients with ACA and in 32% (nine of 28) of patients with LGC carcinoma. Using somewhat higher dosages, Davis et al (14) treated 38 patients with ACA and LGC carcinoma who had received no prior chemotherapy, yet they were able to obtain regressions in only approximately 5% of patients.

With the MACC regimen, given every 3 weeks versus every 4 weeks as in our study, Chahinian et al (2) obtained regression probabilities in 58% (15 of 26) of patients with ACA and in 35% (seven of 20) of patients with LGC carcinoma. Vogl et al (15), however, obtained only a 6% (one of 16 patients) regression probability in ACA with MACC.

The results of this study are consistent with most of those results previously reported. On the CAP regimen, we noted regressions in 13 of 37 patients (35%) with

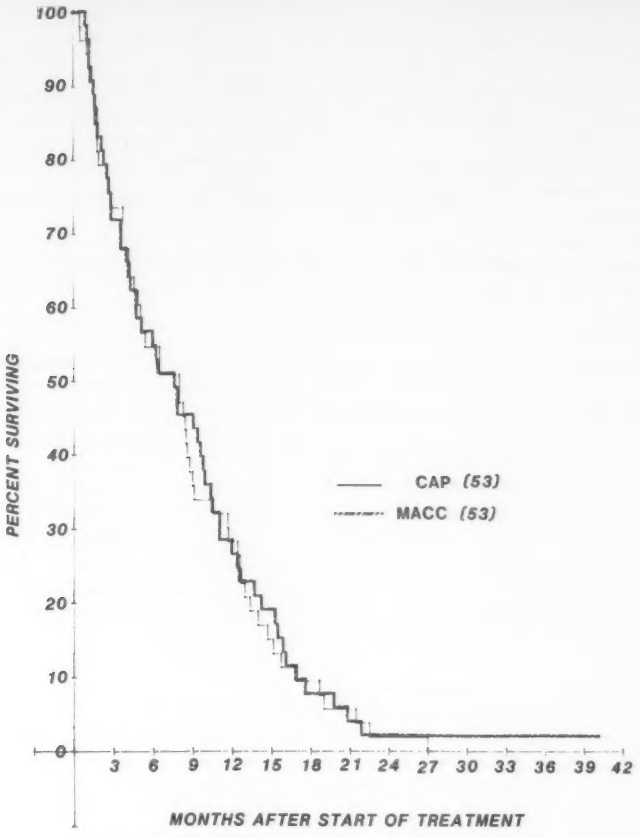

FIGURE 2.—Survival following initiation of the MACC and CAP regimens.

TABLE 3.—Hematologic toxicity

	Treatment regimen	
	CAP	MACC
Leukopenia (cells/mm^3)		
1st course only		
Median nadir (range)	3.5 (1.1–8.5) × 10^3	3.4 (0.6–9.9) × 10^3
Wbc count nadir		
⩽ 4000	70%	67%
< 3000	36%	41%
< 2000	14%	20%
< 1000	0%	4%
All courses		
Wbc count nadir ⩽ 4000	88%	86%
Thrombocytopenia (cells/mm^3)		
1st course only		
Median nadir (range)	226 (92–460) × 10^3	220 (53–601) × 10^3
Platelet count nadir		
⩽ 130,000	10%	22%
< 100,000	2%	8%
< 75,000	0%	6%
< 50,000	0%	0%
All courses		
Platelet count nadir ⩽ 130,000	43%	56%

ACA and in six of 16 (37%) with LGC carcinoma. Following treatment with MACC, we observed regressions in eight of 33 patients (24%) with ACA and in ten of 20 (50%) with LGC carcinoma. These data would tend to support the results of Chahinian et al (2).

The second intent of this randomized trial was to compare the CAP and MACC regimens with respect to regression probabilities, times to disease progression, survival, and toxicity. Regression probabilities (36% vs 34%) were nearly identical, as were distributions of time to disease progression and survival time. On the MACC and CAP regimens, median times to progression were 3.9 and 2.9 months, respectively, while median survival times were 7.5 and 7.9 months. The two regimens produced similar myelosuppression, but those patients receiving MACC did suffer less severe nausea and vomiting, which were the major subjective toxic effects from both regimens. As a result, it appears the MACC regimen can deliver comparable therapeutic efficacy to that of the CAP treatment in patients with ACA and LGC lung cancer.

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Phase I Trial of Intermittent High-Dose Dacarbazine[1]

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A phase I study was conducted with dacarbazine (DTIC) protected from light and administered iv as a single dose every 3 weeks. Eighteen patients received 47 courses of DTIC, with doses ranging from 850 to 1980 mg/m^2. Hypotension was the dose-limiting toxic effect and it may be secondary to the citric acid present in the pharmaceutic preparation of DTIC. Sporadic myelosuppression was seen at doses > 1380 mg/m^2. Other side effects noted were nausea and vomiting, acute diarrhea, headache, a "flu-like" syndrome, and a hypersensitivity reaction to sunlight. No antitumor activity was found. The results of this study indicate that this may be a qualitatively different way of giving DTIC, and that the side effects of this drug may be intermingled with those of citric acid in this particular schedule. If the conventional pharmaceutic preparation of this drug is not modified, further studies with high-dose DTIC protected from light should be discouraged. [Cancer Treat Rep 68:499–504, 1984]

Dacarbazine (DTIC) is an antineoplastic agent synthesized in 1959 in a program designed to develop inhibitors of the biosynthesis of nucleic acids. This compound is an analog of 5-amino-imidazole-4-carboxamide, an intermediate metabolite in the "de novo" synthetic pathway of the purine ring. Although its mechanism of action has not been fully elucidated, the methylation of DNA is probably responsible for the activity of DTIC. In vivo, DTIC is demethylated by liver microsomes with subsequent generation of carbonium ions which are responsible for the alkylation of nucleic acids. When exposed to light, DTIC decomposes rapidly into a diazo-derivative, whose contribution to antitumor activity and side effects of the original compound is not known (1–3).

In the mouse, DTIC has shown antitumor activity against transplanted Sarcoma 180, Carcinoma 755, Lymphosarcoma P1798, and Leukemia L1210, but was minimally effective or inactive against B16 Melanoma, Lewis lung carcinoma, and Ridgway osteogenic sarcoma. It showed no schedule dependence against Leukemia L1210 (1,4).

Clinical phase I and II trials have been performed with various drug schedules and, in most cases, DTIC is administered iv in doses that range from 200 to 400 mg/m^2 for 5 consecutive days, with courses repeated every 3–4 weeks. Reported side effects with this schedule include nausea and vomiting that diminish after subsequent doses, leukopenia and thrombocytopenia, pain along the injected vein, and a "flu-like" syndrome (5). DTIC has shown objective antitumor activity against human tumors, and it is extensively used in combination with other cytotoxic agents in the therapy of metastatic malignant melanoma, soft tissue sarcomas, and Hodgkin's disease (6).

Single doses of 850 or 1000 mg/m^2 of DTIC given every 3–4 weeks appear to be as effective as the conventional 5-day schedule against advanced malignant melanoma, with no increase in toxicity (7,8). However, there is limited information about either the toxicity of higher doses or the maximum tolerable dose of DTIC administered intermittently in large single doses (9). Here we report the results of a phase I study designed to further elucidate those points.

MATERIALS AND METHODS

Patient Selection

All patients eligible for this study had to fulfill the following criteria: (*a*) histologically proven solid tumor refractory to conventional therapeutic regimens; (*b*) Karnofsky index of ⩾ 50%; (*c*) no chemotherapy or radiation

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[3]We thank the nursing staff of the Chemotherapy Department for their skillful assistance during this study.

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therapy administered within 4 weeks before the first course of therapy; (*d*) no prior therapy with DTIC; (*e*) adequate bone marrow (leukocytes $\geq 4000/mm^3$ and platelets $\geq 120{,}000/mm^3$) and renal (serum creatinine < 1.5 mg/dl) and hepatic (serum total bilirubin < 2 mg/dl) functions; and (*f*) informed written consent.

Patients with active infection, CNS metastases, overt psychosis, severe cardiovascular disease, or age > 70 years were excluded from the study.

Treatment Schedule

Commercially available DTIC marketed by Dome/Hollister-Stier (Division of Miles-Martin) was used in the study. DTIC vials of 100 mg (quantitative composition: DTIC, 100 mg; citric acid, 100 mg; and mannitol, 50 mg) or 200 mg (quantitative composition: DTIC, 200 mg; citric acid, 200 mg; and mannitol, 75 mg) were quickly reconstituted in sterile normal saline. All doses were diluted in 200 ml of normal saline and administered over 10 minutes. Both bottle and line had been completely wrapped with tinfoil. The vein injected was infused with 200 ml of normal saline over 15 minutes starting immediately upon the administration of the dose.

When hypotension (defined as a drop in systolic blood pressure to values of ≤ 80 mm Hg) was noted as a side effect, the infusion period was increased to 20 minutes and patients had blood pressure monitoring every 5 minutes for 2 hours after the administration of the dose. Our hypothesis was that this side effect was caused by citric acid through chelation of calcium ions (*see* Discussion section). Consequently, after the occurrence of the second hypotensive episode, every patient treated with DTIC received 5 ml of a 10% solution of calcium gluconate (each milliliter containing 9 mg of calcium ion) at 0, 10, and 20 minutes from the beginning of the treatment. Twenty-five milliliters of the same calcium gluconate solution was added to 500 ml of normal saline that was ready to be infused, in case systolic blood pressure dropped to values of ≤ 80 mm Hg after therapy.

All patients were admitted to the hospital to receive the first course. The initial dose was 850 mg/m^2 and each patient was treated with only one dose level. No dose escalation was performed until patients at the prior dose level had been evaluated for at least 3 weeks. Therapy was repeated every 3 weeks if the patient had recovered from the toxic effects of the preceding cycle.

Study Parameters

All patients had pretreatment history, physical examination, cbc and platelet count, blood chemistries (BUN, uric acid, serum creatinine, calcium, phosphorus, alkaline phosphatase, SGOT, SGPT, gamma glutamyl transpeptidase, total bilirubin, and serum protein), and urinalysis. During the study, cbc and platelet count, blood chemistries, and urinalysis were obtained weekly.

Tumor measurements were performed every 3 weeks. The criteria followed for evaluation of response to therapy and evaluation of toxicity were recommended by the World Health Organization (10).

DTIC was administered for at least two courses in the absence of progressive disease or excessive toxicity, and treatment continued if stable disease was observed.

RESULTS

From January to September 1982, 18 patients were entered in this study. All patients were evaluable. There were four females and 14 males, with a median age of 56 years (range, 32–66) and a median Karnofsky index of 60 (range, 50–80). Histologic diagnoses were lung carcinoma in ten patients (five with squamous cell, two with large cell, two with small cell, and one with adenocarcinoma), colon carcinoma in four, head and neck epidermoid carcinoma in three, and malignant mesothelioma of the peritoneum in one.

All patients had received prior chemotherapy with a median number of two drugs (range, one to seven) and seven had been treated with radiation therapy.

The escalation schedule followed is presented in table 1. The total number of courses administered was 47, with a median of two (range, one to seven per patient). Weekly blood cell counts were available in 35 courses (75%) and only patients in those courses were considered evaluable for myelotoxicity.

Myelotoxicity (table 2) was noted in four patients with doses > 1380 mg/m^2 (two with a dose of 1500 mg/m^2), and its severity was not clearly related to the dose given. One patient treated with a dose of 1650 mg/m^2 had thrombocytopenia associated with normal leukocyte values in two courses. The nadir of leukocytes (Days 14, 18, and 21) and platelets (Days 14, 14, 18, 18, and 21) varied from patient to patient and, except for one patient discussed below, recovery to normal values occurred 7 days later. No cumulative myelosuppression was observed in three patients who received more than three cycles. There was no systematic search for eosinophilia, and no significant changes in hemoglobin values were observed.

TABLE 1.—DTIC dose levels

Dose (mg/m^2)	No. of patients	No. of courses
850	2	4
1000	2	4
1200	3	8
1380	3	15
1500	3	6
1650	3	6
1980	2	4
Total	18	47

TABLE 2.—Myelotoxicity

Dose (mg/m^2)	No. of evaluble courses	Leukocyte count*				Platelet count*			
		0	1	2	3	0	1	2	3
850	4	4	-	-	-	4	-	-	-
1000	3	3	-	-	-	3	-	-	-
1200	6	6	-	-	-	6	-	-	-
1380	8	8	-	-	-	8	-	-	-
1500	5	3	-	1	1	3	1	-	1
1650	5	5	-	-	-	3	1	1	-
1980	4	3	-	-	1	3	-	-	1

*Grading (values × 10^3 cells/mm^3): 0 = leukocyte count ⩾ 4.0, platelet count ⩾ 100; 1 = leukocyte count, 3.0–3.9, platelet count, 75–99; 2 = leukocyte count, 2.0–2.9, platelet count, 50–74; and 3 = leukocyte count, 1.0–1.9, platelet count, 25–49. Values in table = No. of courses.

One patient with extensive prior chemotherapy (seven drugs) presented 1100 leukocytes/mm^3 and 47,000 platelets/mm^3 on Day 14 of the second cycle after receiving uneventfully the first course of DTIC at a dose of 1980 mg/m^2. He refused admission to the hospital and died at home on Day 21, possibly because of sepsis.

Toxic effects other than myelotoxicity are presented in table 3. Nausea and vomiting appeared in 90% of the courses, and acute diarrhea occurred in 89% of the cycles with doses of DTIC > 1200 mg/m^2. The diarrhea consisted of uncontrollable emission of 3–4 watery stools 30 minutes after the completion of drug infusion which subsided without specific therapy within 2 hours.

A flu-like syndrome was reported in 40% of evaluable courses, and discrete pain along the vein injected was noted in 43% of the cycles. Tension headache with "pin-like" itching, associated or not associated with perioral paresthesias, was noted during drug infusion in 37% of the courses. No phlebitis or fever related to drug administration was observed, and there was one case of accidental extravasation of drug without the appearance of any kind of local reaction.

A reaction to sunlight exposure occurred in seven of 26 evaluable cycles administered in our outpatient clinic (table 3). Patients leaving the clinic shortly after administration of DTIC experienced an unbearable burning of sun-exposed areas (usually head and hands) that disappeared when they went into shady areas. No erythema or edema was observed after the reaction.

The patients did not present alopecia, stomatitis, or changes in hepatic or renal function parameters. Urine had a brick color about 8 hours after therapy in all patients treated with doses of DTIC > 1200 mg/m^2.

Two patients had blurred vision during the infusion of 1380 mg/m^2 of DTIC. One patient receiving a dose of 1500 mg/m^2 and a second patient treated with a dose of 1650 mg/m^2 developed hypotension (table 4), followed by shock and loss of consciousness 5 and 20 minutes, respectively, from the completion of drug infusion. During the hypotensive episode, the patient treated with a dose of 1650 mg/m^2 had generalized convulsions without loss of sphincter control. In both cases, systolic blood pressure dropped to values as low as 40 mm Hg and returned to normal values 40 minutes later with the administration of plasma expanders and corticosteroids. Those patients had no changes in serum total calcium, magnesium, or in the percent of calcium bound to protein up to 4 hours from the end of DTIC infusion. Because serum citrate values were not determined, availability of serum-ionized calcium could not be estimated. The duration of the infusion was increased to 20 minutes after those episodes.

Two additional patients treated with a dose of 1650 mg/m^2 and two patients treated with a dose of 1980 mg/m^2 received calcium gluconate during and after the infusion of the drug (*see* Materials and Methods section). Those patients had monitoring of blood pressure after treatment for 2 hours. The first two patients had calcium gluconate infusion started immediately after the comple-

TABLE 3.—Nonhematologic toxicity*

Dose (mg/m^2)	Nausea	Vomiting	Diarrhea	Pain during injection	Flu-like syndrome	Headache	Reaction to sunlight	Hypotension
850	1/4	3/4	1/2	3/4	—†	0/1	0/1	—
1000	2/4	2/4	0/2	1/4	—	1/3	0/1	—
1200	1/7	6/7	0/3	1/6	3/6	1/7	—	—
1380	15/15	15/15	12/14	4/11	0/15	7/15	3/11	—
1500	6/6	6/6	6/6	3/6	6/6	2/5	2/5	1/6
1650	3/3	3/3	4/4	1/4	2/2	1/2	0/4	1/6
1980	4/4	4/4	3/4	4/4	2/3	1/2	2/4	4/4

*Values = No. of occurrences/No. of evaluable courses for each particular side effect.
†Data not recorded.

TABLE 4.—Hypotension and dose of citric acid (DTIC)*

Patient No.†	DTIC dose (mg/m^2)	Citric acid (DTIC) administered (mg)	Hypotension‡
11	1500	2730	1/1§
12	1500	2370	0/3
13	1500	2370	0/2
14	1650	2772	1/1§
15	1650	2673	0/3
16	1650	2600	0/2
17	1980	2732	2/2
18	1980	3128	2/2

* Marketed DTIC contains equal amounts (mg by mg) of DTIC and citric acid.

†Patients 15–18 received calcium gluconate (*see* the Materials and Methods and the Results sections).

‡Values = No. of episodes/No. of cycles. Hypotension = systolic blood pressure ⩽ 80 mm Hg.

§Systolic blood pressure < 50 mm Hg.

tion of DTIC therapy, and they maintained values of systolic blood pressure > 80 mm Hg, with a decrease < 20 mm Hg from basal values. Both patients had tachycardia (⩽ 120 beats/minute) that persisted for 2 hours. Two patients at the highest dose level had tachycardia (⩽ 160 beats/minute) up to 3 hours after DTIC therapy, with a decrease in their systolic blood pressure to lower median values of 70 mm Hg that recovered to basal figures with the infusion of calcium gluconate. In figure 1, we present serial blood pressure determinations in one patient who received two courses of 1980 mg/m^2 of DTIC. This illustrates the recovery in blood pressure values with the infusion of calcium gluconate.

Measurable disease was present in seven patients; there was no measurable disease in 11 patients. No objective antitumor activity was detected during the study.

DISCUSSION

The results of this study indicate that when commercially available DTIC is administered iv and protected from light in a single dose every 3 weeks, hypotension represents the dose-limiting toxicity.

Hypotension has not been previously reported as a side effect associated with DTIC therapy and, although its causes are not definitely established, our hypothesis is that it may be related to concomitant administration of citric acid. This compound is added to the pharmaceutic preparation of DTIC in order to provide mild acidic conditions that favor drug solubility (*see* Materials and Methods section).

The sodium salt of citric acid is a well-known chelating agent for calcium ions, and it is commonly used as a blood preservative. It removes ionized calcium from plasma and thereby prevents clotting. Iv administration of 3.8–6.9 g of sodium citrate to man over 10–20 minutes has been associated with moderate to severe cardiac depression and hypotension. This hypotension correlated with a decrease in serum ionized calcium to values as low as 0.50 mmols/L (normal range, 1.12–1.40). Hypotension was reversed by stopping the infusion of sodium citrate or by the administration of calcium chloride (11).

The schedule chosen for our study exposed patients to high doses (median, 2.2 g; range, 1.40–3.10) of citric acid infused over a short period of time. In every case, patients received exactly the same amount of DTIC and citric acid. After the first two hypotensive episodes observed with 1500 and 1650 mg/m^2 of DTIC, respectively (table 4), iv administration of calcium gluconate prevented or effectively corrected (fig 1) further episodes. These data suggest that citric acid may be responsible for the hypotensive episodes observed in our study.

Cowan and Bergsagel (9) reported a phase I clinical trial with an intermittent DTIC schedule. They treated 20 patients with doses that ranged from 650 to 1450 mg/m^2 of DTIC, with no special precautions mentioned to protect DTIC from light. The toxic effects encountered (percent of patients) consisted of nausea (100%), diarrhea (45%), vomiting (60%), fever for 24 hours (30%), and a flu-like syndrome associated with fever (70%). Granulocytopenia and thrombocytopenia were consistently noted with increasing frequency at doses > 1000 mg/m^2. Five of six patients treated with a dose of 1450 mg/m^2 had < 1000 granulocytes/mm^3, and three of those six had < 100,000 platelets/mm^3. The authors (9) do not report on hypotensive episodes.

In our study, toxicity other than hematologic was similar to that reported by Cowan and Bergsagel, except that we had no patient with fever associated with drug therapy. However, myelosuppression was less marked in our patients treated with equivalent doses. Leukopenia associated or not associated with thrombocytopenia was noted with three different dose levels (table 2) and its severity did not increase proportionally with the dose given. No significant differences between patients were encountered to explain this finding, although the number of patients treated is too small to draw definitive conclusions. Slight individual variations in the reconstitution of

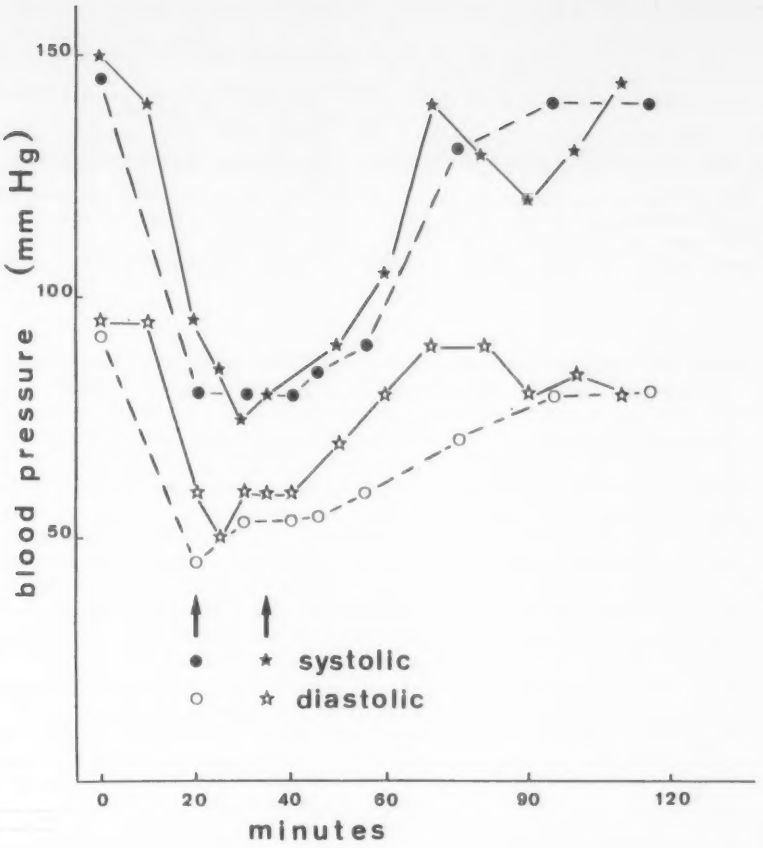

FIGURE 1.—Patient 17 received 2 cycles with 1980 mg/m^2 of DTIC infused over 20 mins, and 5 ml of a 10% solution of calcium gluconate was injected iv at 0, 10, and 20 mins. When systolic blood pressure values dropped to 80 mm Hg, he was infused with a solution of 25 ml of the same calcium gluconate solution in 500 ml of normal saline (arrows). Closed symbols = systolic values; stars = 1st cycle.

the vials could have, in some cases, facilitated a higher light exposure of the drug, with partial decomposition of DTIC and a higher toxicity.

Beck et al (12) recently reported two patients who had a photosensitivity reaction following the administration of 250 and 300 mg/m^2 of DTIC, respectively. The patients had intense erythematous rash in sun-exposed areas that lasted up to 24 hours and recurred after the administration of the daily DTIC dose. The rash could be prevented by limiting sun exposure. This photosensitivity reaction was less marked in our patients. None had erythema and the reaction was not noted 2-3 hours after drug administration.

From our data, we conclude that administration of DTIC protected from light is a qualitatively different way of giving this drug, with lower toxicity on bone marrow, although the therapeutic significance of this finding is unknown at the present. Citric acid added in the pharmaceutic preparation of the drug may be responsible for the hypotensive episodes noted, which were dose-limiting, but other side effects reported could as well be attributable to this compound.

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Pharmacokinetics of Free and Total Platinum Species After Short-Term Infusion of Cisplatin[1]

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Pharmacokinetic studies were performed in ten patients who received short-term (4–15-min) infusions of cisplatin. Computerized nonlinear least-squares analysis (NLIN) and an adapted curve-stripping approach (CSTRIP) were used to characterize total-platinum concentration-time curves. The overall curves showed a rapid initial phase and a prolonged terminal phase, separated by a phase with secondary peaks attributed to the existence of an enterohepatic recirculation. Up to Day 5, NLIN analysis revealed three exponential phases, with half-lives of 14.4 mins, 273.7 mins, and 5.3 days, respectively. However, a significant consistent divergence ($P < 0.005$) was found between the observed and predicted curves during the intermediate phase, not justifying the use of an exponential function during this phase. The shape of the intermediate curve was strongly suggestive of a second influx in the plasma compartment, the amount of which was estimated by the CSTRIP approach (1.4% ± 0.5% of the administered dose). Free-platinum levels declined in a biphasic manner (half-lives: 9.7 ± 0.2 and 40.4 ± 2.5 mins; n = 3). After administration of 100 mg/m² of cisplatin, maximum platinum levels in rbcs ranged from 0.51 to 0.58 μg/ml and were reached within 90–150 mins. Thereafter, rbc platinum levels declined in a biphasic fashion, with a terminal half-life, for the interval Days 5–15, of 36–47 days. The binding of platinum to both plasma and proteins and rbcs in vitro (using patients' own blood) was slow, biphasic, and irreversible. [Cancer Treat Rep 68:505–513, 1984]

The antineoplastic agent cisplatin is active against a variety of tumor types (1–6). Many pharmacokinetic studies have analyzed the kinetics of cisplatin in man (7–29), but in most of them the observation period was limited to 2 days and free (unbound) platinum concentrations were not determined. Plasma clearance of total platinum after single doses of cisplatin has generally been reported to be biphasic, but in a limited number of reports three or four exponential terms have been described (11,13,28,29). The major pathway of excretion of platinum species is via the urine. However, limited amounts are excreted via the bile (23). In an earlier report we suggested the existence of an enterohepatic recirculation (EHC) in one patient (24).

In the present study cisplatin was administered by short-term infusion (4–15 mins) and was given in either low doses (40–50 mg/m²) or a high dose (100 mg/m²). The purpose of the study was to evaluate the pharmacokinetics of free and total platinum, and additionally to obtain pharmacokinetic evidence of an EHC. The patients' own blood was used separately for in vitro studies performed to investigate the binding of cisplatin to plasma proteins and rbcs.

MATERIALS AND METHODS

Patients

Ten patients (seven males, three females) were entered in the study. All patients received cisplatin as a single agent, and all but one had never received cisplatin before. The diagnoses were head and neck cancer (eight patients), urethral cancer (one patient), and ovarian cancer (one patient).

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Seven patients (Nos. 1-7, table 1) had a normal renal function (serum creatinine < 1.35 mg/100 ml, creatinine clearance ≥ 65 ml/min/1.73 m^2), a normal hepatic function, and no signs of ascites or pleural effusion. The mean age of this group of patients was 58 yrs (range, 42-74).

The remaining three patients formed a separate group. Patient 8 (59 yrs of age) had an increase in serum creatinine prior to treatment (1.58 mg/100 ml) and had an ureterointestinal anastomosis. Patient 9 (69 yrs of age) received a prolonged posthydration scheme (2 L/24 hrs; Days 2-5). Patient 10 (48 yrs of age) had received two courses of cisplatin before and had been treated for a septic shock during the second treatment course. Serum creatinine was still within the normal range, but creatinine clearance (65 ml/min/1.73 m^2) was 50% of its original value.

Study Design

All patients received cisplatin prepared from vials of Platinol. After reconstitution with sterile water the solution containing 1 mg/ml of cisplatin was used immediately after preparation. Dosage and duration of infusion are specified for each patient in table 1.

All patients but one received 1 L of normal saline during the 4-hr period preceding cisplatin administration, and 4 L of saline over the 24-hr period following drug administration (Patient 3 received 5% dextrose). Mannitol and furosemide were not used on a routine basis and none of the patients received one or both diuretics before, during, or within 6 hrs after cisplatin administration.

Blood of two patients (Nos. 2 and 3) was sampled (80 ml) prior to administration of cisplatin for in vitro binding experiments. Blood samples of all patients were drawn into heparinized tubes prior to administration of cisplatin, at the end of infusion, and 5, 10, 15, 30, 60, 90, 120, 150, 180, 210, and 240 mins and 6, 8, 21, and 24 hrs after infusion. Thereafter, blood samples were taken daily (24-hr intervals) for at least 1 week. Urine collection was started just before administration of cisplatin; 6-hr samples were collected during the first 24-hr period, while thereafter, 24-hr samples were collected for at least 1 week.

All blood samples were centrifuged immediately and the plasma was removed. For three patients (Nos. 1-3) 4-ml plasma samples were filtered through Amicon Centriflo CF50A cones and the ultrafiltrate fraction of 600-1100 μl was used for the determination of the free-platinum concentration. Rbcs of the same patients were washed twice with an equal volume of normal saline and centrifuged at 1000 × *g* for 10 mins. Rbcs, plasma, ultrafiltrate, and aliquots of urine samples were stored at -30°C until analysis.

Platinum Analysis

All samples were thawed just before analysis and diluted (1:1 vol/vol for plasma and 9:1 vol/vol for ultrafiltrate and urine) with a solution containing 0.4 N HCl and 0.15 M NaCl for plasma and 0.6 M NaCl in 2 N HCl for urine and ultrafiltrate. Thawed rbcs were disrupted in an ultrasonic bath for 10 mins. An 0.5-ml aliquot of lysate was digested by 3 ml of 65% HNO_3 at 170°C in a polytetrafluoroethylene (PTFE) bomb for 2 hrs. The remaining liquid was evaporated at 120°C under a stream of air after addition of 5 mg of NaCl. The residue was dissolved in 1 ml of 0.2 N HCl-0.15 M NaCl.

The concentration of platinum in all samples was deter-

TABLE 1.—Comparison of pharmacokinetic parameters by nonlinear least-squares analysis (NLIN) f

Patient No.	Infusion time (mins)	Cisplatin dose (mg/m^2)	Elemental platinum (mg)	NLIN Intercepts (μg/ml)* A	B	C	Half-life† $t_{1/\alpha}$ (mins)	$t_{1/\beta}$ (mins)	$t_{1/\gamma}$ (days)
1	7.5	100	85.8	5.12	0.93	1.62	16.8	126	5.6
2	7.8	100	146.3	6.50	0.94	1.62	11.1	108	5.9
3	6.0	100	136.5	9.32	0.80	2.30	11.0	409	4.2
4	15.0	100	113.8	7.04	0.63	1.91	18.2	689	6.5
5	4.3	40	48.1	4.22	0.55	0.85	8.2	119	4.1
6	4.2	40	46.8	1.71	0.37	0.88	18.7	112	5.4
7	4.2	40	46.8	2.49	0.27	0.66	16.7	353	5.6
Mean ± SD							14.4 ± 4.2		5.3 ± 0.9
8	10.0	50	55.6	2.27	2.33	1.28	2.8	33	4.5
9	5.3	100	117.0	6.62	3.07	2.20	3.1	45	3.0
10	8.0	40	45.5	1.42	0.28	0.72	24.2	212	6.1

*Intercepts (I) corrected for infusion time ($I = I^* \cdot T/(1-e^{-slope \cdot T})$).

†$t_{1/2} = -\frac{0.0693}{slope}$.

‡V_D^{ext} = apparent volume of distribution (dose/B).

§Correlation coefficients ranged from -0.950 to -0.999.

¶Correlation coefficients ranged from -0.916 to -0.997.

mined by flameless atomic absorption spectrometry. The precision of the assay (C.V.) was about 3% in the concentration range of 0.5–3.0 μg of Pt/ml.

In Vitro Studies

The binding of cisplatin to plasma proteins and the rbcs was studied separately in vitro. One milliliter of a cisplatin solution containing 2.1 mM cisplatin (≃ 400 μg of Pt/ml), 0.4 N HCl, and 0.15 M NaCl was added to 40 ml of plasma of each of the two patients. The incubation was performed under mild shaking in the dark at 37°C for 24 hrs. Four-milliliter aliquots were drawn at 0, ½, 1, 2, 4, 8, and 24 hrs. All aliquots were ultrafiltered immediately after withdrawal. The total-platinum concentration was determined at the start and after 24 hrs of incubation.

Release of platinum from plasma proteins was studied with a Dianorm apparatus with 24 cells. Each cell consisted of two PTFE half-cells of 1 ml each, separated by a spectrapor membrane (cut-off, 12,000–14,000 daltons). One half-cell was filled with 1 ml of plasma and the other with 1 ml of plasma preincubated with a cisplatin solution for 24 hrs at 37°C. The cells were incubated at 37°C under rotation. The platinum content was analyzed in both half-cells of two cells withdrawn daily for 8 days.

Binding of cisplatin to rbcs was studied by mixing 7 ml of rbcs with 7 ml of 0.05 mM cisplatin (≃ 10 μg Pt/ml) in 0.15 M NaCl. The incubation was performed under mild shaking in the dark at 37°C for 24 hrs. One-milliliter aliquots were withdrawn at 0, ¼, 1, 2, 4, 8, and 24 hrs. All aliquots were centrifuged immediately after withdrawal. The supernatants were separated for determination of the platinum concentration.

Method of Data Analysis

Curves of total-platinum concentration versus time in plasma were studied in two different ways. Computerized curve-fitting was performed with the NLIN method of Marquardt (30) as implemented by Hewlett-Packard for the HP 9845A. The second method used was CSTRIP, which was applied so that the linear least-squares fit of the concentrations on Days 1–5 was subtracted from the first part of the curve, followed by a linear least-squares fit of residual concentrations during the first 0–15 mins (C_p^t-C_p^β). Next, the residuals of the remaining intermediate phase (15 mins–24 hrs) were also diminished by the extrapolated values of the first phase. The final residuals (C_p^t-C_p^β-C_p^α) obtained by this procedure were analyzed separately.

RESULTS

Clinical Pharmacokinetics

A semilogarithmic plot of the total-platinum concentrations in plasma versus time is shown for all patients in figure 1. All curves (except that of Patient 8, with limited sampling) showed positive deviations between 2 and 8 hrs after administration, and in six patients a small peak was observed 3–4 hrs after the end of the infusion. This phenomenon indicated a physiologic cause and could be explained by a second influx of platinum into the plasma compartment. With the NLIN analysis, a triexponential curve was found to best fit total-platinum levels for all patients over the first 5 days. The corresponding calcu-

...s of the concentration curve up to 5 days and by curve-stripping analysis (CSTRIP) for the elimination and rapid disposition phase

SD of observation (%)	CSTRIP					
	Intercepts (μg/ml)*		Half-life†			
	A	B	$t_{½\alpha}$§ (mins)	$t_{½\beta}$¶ (days)	V_D^{ext}‡ (L)	EHC (% of dose)
3.5	6.63	1.82	18.5	5.4	47.1	0.7
5.3	8.28	1.55	13.7	7.2	94.4	1.0
5.7	11.11	2.29	11.9	4.2	59.6	1.8
2.7	8.70	2.04	19.2	5.6	55.8	1.1
9.5	5.59	0.83	8.4	4.4	61.7	1.5
4.6	2.06	0.89	24.0	5.4	52.6	2.2
3.3	3.09	0.67	15.8	5.8	69.8	1.5
			15.9 ± 5.2	5.4 ± 1.0	61.9 ± 16.7	1.4 ± 0.5
4.7	5.23	1.27	11.7	4.6	43.8	0.5
5.3	8.95	1.97	9.9	3.6	59.4	2.3
5.3	2.07	0.64	22.0	8.2	71.1	1.5

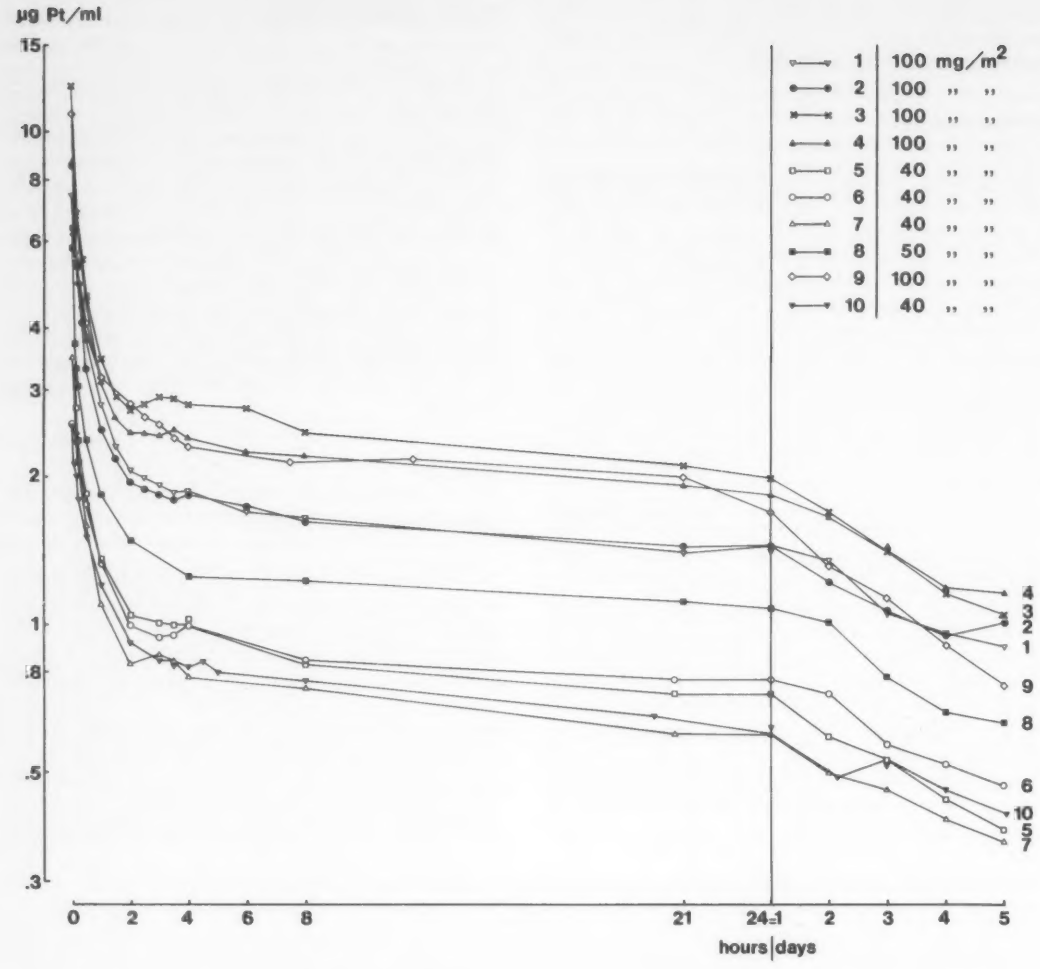

FIGURE 1.—Semilogarithmic plots of total-platinum concentrations in plasma vs time in 10 patients given cisplatin by rapid iv infusion.

lated pharmacokinetic parameters are summarized in table 1. The use of an exponential function during the second phase became questionable because of consistent deviations from the model during this phase (β-half-life × 5 = 1370 mins), and was beyond the influence of the α-phase (α-half-life × 5 = 70 mins). As a rule, the ratios of the observed and predicted concentration between 90 and 150 mins after administration were < 1 (table 2), and this was significant at 120 mins (ANOVA, $P < 0.005$). The shape of the curve obtained by the final residuals superposed on the second exponential phase, showing an early peak, was strongly suggestive of a second influx of platinum in the plasma compartment during this phase. Because of this, no justification could be found for a straightforward use of the NLIN model. Therefore, the CSTRIP method was used as described under the Method of Data Analysis section. With this approach the basic pharmacokinetic parameters were calculated without assumptions about the character of the intermediate phase (table 1). From the NLIN model it could be deducted that the elimination phase was unaffected by the intermediate phase from Day 1, while the interval 0–15 mins for the calculation of α-half-life was based on maximal correlation coefficients. As expected, an excellent agreement was found between B and β-half-life calculated by CSTRIP and C and γ-half-life calculated by NLIN. The α-half-lives calculated by CSTRIP are only slightly higher than the values found with NLIN because

TABLE 2.—Ratio of observed and predicted concentrations between 30 and 1440 mins with a triexponential NLIN analysis of the curve for the first 5 days

Patient No.	Time-point of measurement (mins)											
	30	60	90	120	150	180	210	240	360	480	1260	1440
1	0.996	1.050	1.006	0.974	0.983	0.998	0.987	1.020	1.000	1.014	0.962	1.005
2	1.016	1.049	1.016	0.960	0.969	0.977	0.979	1.027	1.048	1.016	0.982	1.003
3	1.069	1.084	0.938	0.932	0.970	1.021	1.025	1.003	1.046	0.971	1.012	0.979
4	1.013	0.999	0.998	0.978	1.002	1.005	1.041	1.003	0.975	0.995	1.001	0.981
5	1.013	1.019	0.936	0.968	0.984	1.007	1.026	0.999	—	0.995	1.016	1.016
6	1.015	1.012	0.985	0.924	0.972	0.946	0.990	1.043	0.948	0.982	0.974	0.991
7	0.992	1.005	0.993	0.942	0.970	1.037	1.018	0.958	1.010	1.002	0.975	0.983
Mean	1.016	1.031	0.982	0.954	0.978	0.999	1.009	1.008	1.005	0.997	0.989	0.994

of the influence of the intermediate phase. The intermediate part of the curve is obtained by subtracting the extrapolated CSTRIP values of the α- and β-phases (C_p^{α}, C_p^{β}) from the levels measured during the first 24 hrs after administration (C_p^t). Figure 2 shows C_p^t-C_p^{β}-C_p^{α} versus time for all patients. Similar patterns were obtained irrespective of the original shape of the total platinum curve. Peak values of C_p^t-C_p^{β}-C_p^{α} were reached between 0.5 and 4 hrs and ranged between 0.24 and 1.1 μg of Pt/ml of plasma. Again, the appearance of peak values suggested a second influx which, together with the duration of the intermediate phase (about 24 hrs), is an argument in favor of the existence of an EHC. The CSTRIP approach also allowed an estimation of the percentage of the dose contributing to this EHC, ie, from the ratio of the areas under the curve C_p^t-C_p^{β}-C_p^{α} versus time ($AUC_{EHC}^{0\text{-}24\ hr}$) and C_p^t versus time (AUC_p^{∞}). The values obtained ranged from 0.5% to 2.3% (table 1).

In four patients blood samples were obtained for 18 days or longer. NLIN analysis revealed four exponential terms to best fit the total-platinum concentration curves over this observation period. Intercepts, half-life values for the different phases, and standard deviation of observations are summarized in table 3. The terminal half-life in these patients indicates that the rate of elimination is not constant but decreases with time.

Free-platinum concentrations decayed in a biphasic manner. The NLIN fit revealed a half-life of the initial phase of 9.7 mins (range, 9.5–9.8; n = 3), and a mean half-life of the second phase of 40.4 mins (range, 37.5–42.0). Free-platinum concentrations were determined in Patients 1–3, all receiving 100 mg/m^2 of cisplatin. In these patients free-platinum concentrations reached the analytic detection limit 3 hrs after administration. Before that time (90–150 mins after administration), plateau levels of platinum in rbcs were reached (range, 0.51–0.58 μg of Pt/ml). Platinum concentrations in rbcs were followed for 21 and 15 days in Patients 2 and 3, respectively. The decay curves of platinum concentrations in rbcs were best fitted with NLIN by two exponential terms, revealing initial half-life values of 13 and 34 hrs in Patients 2 and 3, respectively, and a half-life value for the second phase of 42 days for Patient 2 and 164 days (due to an outlying concentration on Day 4) for Patient 3. Using linear least-squares analysis, half-life values calculated over the time interval Days 5–15 were 36 and 47 days for Patients 2 and 3, respectively.

Figure 3 illustrates the decay of the platinum concentrations in plasma, plasma ultrafiltrate, and rbcs in one patient (No. 2).

Cumulative urinary excretion of platinum in Patients 1–7 was 24.4% ± 4.6% after 6 hrs. This slowly increased to 41.5% ± 4.7% after 6 days. In Patient 9, cumulative urinary excretion did not differ from that found in the main group of patients. However, in Patient 10 this was reduced, especially during the first 6 hrs (13.9%). After

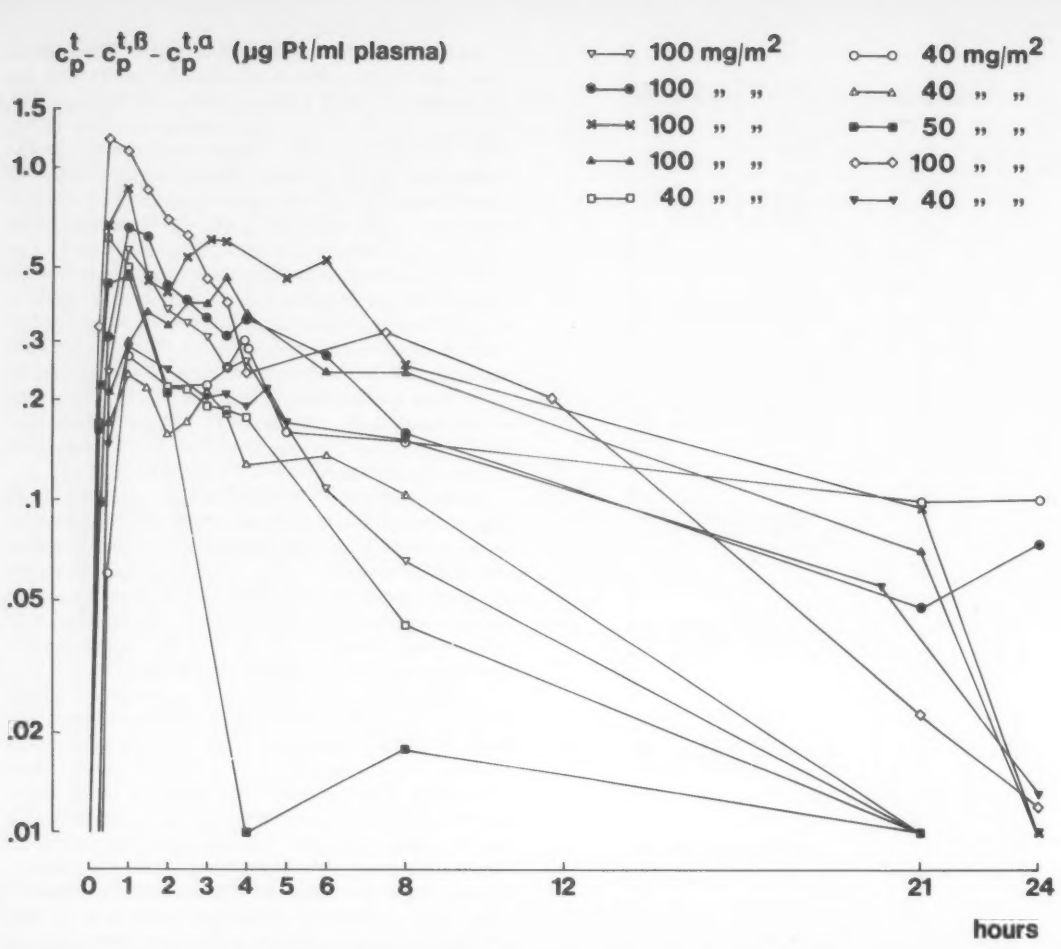

FIGURE 2.—Semilogarithmic plots of the residuals after application of CSTRIP to the concentration-time curves of the 10 patients given cisplatin by rapid iv infusion. (C_p^t = measured total-platinum concentration in plasma at time t; $C_p^{t,\beta}$ = calculated total-platinum concentration in plasma at time t by back-extrapolation of the elimination line; and $C_p^{t,\alpha}$ = calculated total-platinum concentration in plasma at time t by extrapolation of the initial rapid disposition line after subtraction of the elimination line.

the first day the percent increase was in the same range as that found in the other patients.

In Vitro Observations

After addition of cisplatin to the isolated plasma and rbcs of Patients 2 and 3, a biphasic decline of the free-platinum concentrations was observed. This is illustrated in figure 3 for Patient 2. Mean half-life values of the initial phase for binding to plasma proteins and rbcs was 72 and 122.4 mins, respectively. For the second phase of binding to plasma proteins this value was 109.7 for Patient 2 and infinite for Patient 3, while the mean half-life value of the second phase for binding to rbcs was 54.5 hrs (range, 52.6–56.4). After incubation for 24 hrs, the mean value for binding to plasma proteins was 96% and to rbcs was 57%.

Studies on the release of platinum from plasma preincubated with cisplatin for 24 hrs revealed only a 5% loss from the platinum-containing half-cell in 8 days. This indicates that cisplatin was almost irreversibly bound to plasma proteins. Exposure of platinum-loaded rbcs to saline also showed that the binding to rbcs is irreversible.

DISCUSSION

NLIN curve-fitting has generally been accepted for an accurate computation of the parameters of multiple expo-

TABLE 3.—Values of pharmacokinetic parameters obtained by NLIN analysis of concentration-time curves up to the 36th day

Patient No.	Observation period (days)	Intercept (μg/ml)				Half-life*				SD of observations (%)
		A	B	C	D	α (mins)	β (hrs)	γ (days)	∞ (days)	
4	36	6.25	0.75	1.05	0.63	19.2	1952.1	5.2	15.9	5.85
8	18	1.97	2.48	0.51	0.83	1.6	26.5	1.4	9.4	6.53
9	19	5.00	3.43	1.20	1.17	1.9	32.9	1.1	7.4	5.42
10	21	1.45	0.27	0.29	0.38	22.9	357.5	3.6	15.3	6.66

*Half-life = $\frac{0.693}{\text{slope}}$

nential functions (31). With this approach we found an optimal fit with three exponential terms for the concentration-time curves up to Day 5, and at least four exponential terms were found when total-platinum concentration curves were analyzed up to at least 18 days. Accurate comparison of elimination half-lives with those reported by others is hindered by the strong dependence on the duration of the period during which platinum concentrations are measured. However, our observations are in agreement with those of other investigators, who also reported the presence of three or more compartments when the measurements were performed for a longer period (11,13,28). The increase of half-life with time may be explained by the appearance of biotransformation products (32,33), an irreversible binding of platinum species to plasma proteins (33), relating half-lives to the turnover rates of plasma proteins, and the existence of deep compartments. Long half-lives due to the presence of deep compartments mean that, in contrast to the plasma compartment, accumulation of platinum may occur in these

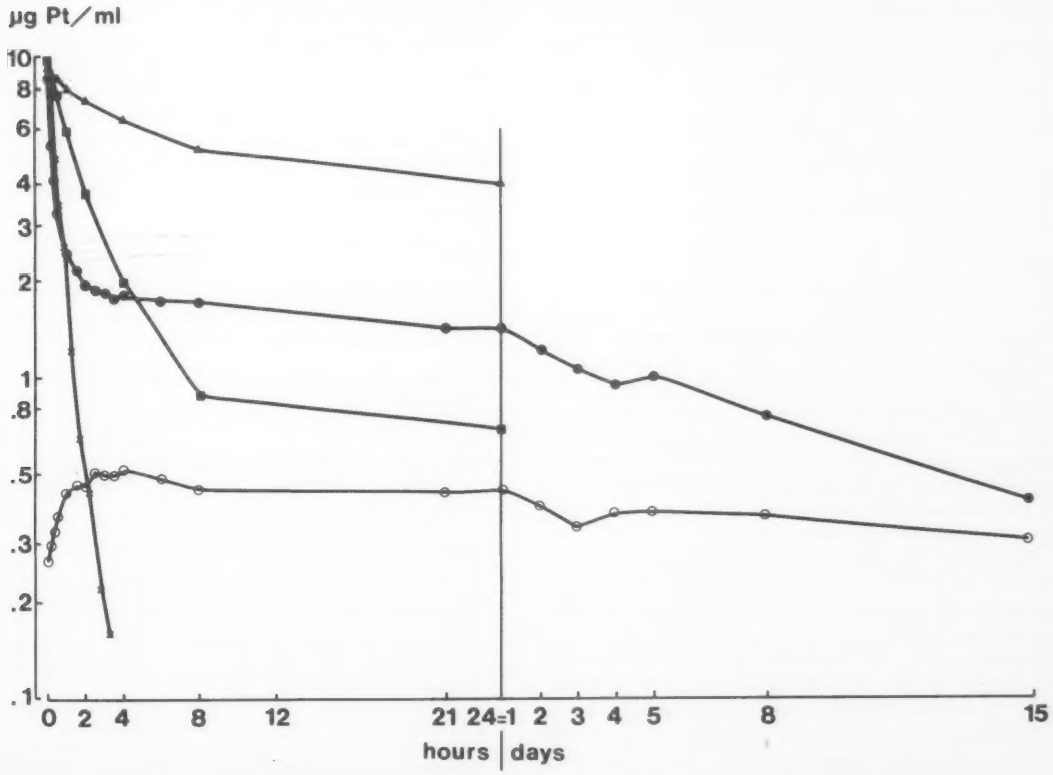

FIGURE 3.—Semilogarithmic plots of the platinum concentrations in plasma in vivo (●-●), in plasma ultrafiltrate in vivo (x-x), in rbcs in vivo (O-O), in plasma ultrafiltrate in vitro (■-■), and in rbcs in vitro (▲-▲) vs time in Patient 2.

tissue compartments with an increasing number of cycles (34). This statement is affirmed by organ distribution studies in dogs (35,36), showing significant platinum levels in tissues even 2 months after a single administration of cisplatin (35). The increasing half-life also indicates that the volumes of distribution mentioned in table 1 and calculated from the intercept of the platinum concentrations during Days 1-5 are too low. Nevertheless, the values retain their meaning as an operational volume of distribution.

Because of the irreversible binding of cisplatin to proteins, the V_D of platinum should be placed in a different perspective. In the first place, V_D should be calculated for the unbound drug. Our in vitro studies revealed that 96% of cisplatin is bound to plasma proteins after 24 hrs. This means that the V_D of free-platinum species ($V_{D,free}$) is at least 25 times greater than that calculated for total platinum. A $V_{D,free}$ of about 1600 L indicates that distribution is not restricted to intra- and extra-cellular fluids but also extends to peripheral compartments, where storage may occur. This is in agreement with the distribution studies in dogs, as earlier mentioned, and is also consistent with the incomplete recovery of platinum in human urine.

Excretion of platinum via the urine is the major pathway of excretion; however, biliary excretion of platinum has also been demonstrated in a patient with a permanent T tube (23). Maximum platinum concentrations in the bile of that patient were reached 1 hr after iv bolus injection of cisplatin and then dropped to undetectable levels at 5 hrs. Studies with radiolabeled cisplatin have indicated limited fecal excretion (10,24), and in one of these studies the presence of an EHC was suggested (10). The manifestation of secondary peaks in the declining concentration-time curve in plasma at 3-4 hrs in six of our ten patients and the deviation of the observed concentrations from the second exponential term obtained with the NLIN fit, together with data from the literature, suggest the presence of an EHC (37). Secondary peaks have also been observed by others (21,22).

Because of these indications, we did not restrict ourselves to the overall NLIN three-exponential fit with a listing of the residuals in the second phase, but tried to isolate the results of the second influx ($C_p^t - C_p^\beta - C_p^\alpha$) by using the described CSTRIP method. This approach also allowed estimation of the extent of EHC.

The biphasic decline of free-platinum concentrations after rapid infusions is in agreement with the data reported by Patton et al (17) and Belt et al (18). In their studies the initial phase was not as pronounced as in our study because of wider time intervals (17) or lack of early measurements after administration (18). For that reason only the terminal half-lives are in very good agreement. It is self-evident that terminal half-life in our study will be longer than that found in studies with a monoexponential approach (25,29). Half-life of free-platinum species is determined by a combination of protein binding, elimination, and transport to tissues. In addition, the terminal half-life might also be influenced by re-entry of platinum into the plasma compartment and metabolism of the original cisplatin (32).

Uptake of platinum into rbcs was very rapid and maximum levels were reached within the period of detectable concentrations of free platinum. The mean maximum platinum level in rbcs (0.54 μg of Pt/ml) was lower than the mean level of platinum bound to proteins at the same time (1.75 μg of Pt/ml). This observation can be clarified by the in vitro experiments in which the uptake of platinum by rbcs was much slower than the binding of platinum to the plasma proteins. The in vitro experiment also showed that after 24 hrs of incubation the concentrations in rbcs and supernatant are almost equal, which indicates the absence of an active transport mechanism and a lack of abundant binding sites in the rbcs. The long final half-life value of platinum in rbcs in vivo is in accordance with the irreversible binding of platinum to rbcs, as postulated from our in vitro studies. The initial rather rapid decrease in rbc platinum concentration needs further elucidation. Our data do not exclude a cisplatin-induced breakdown or a slow release of platinum from rbcs in vivo (38,39).

Patients 8-10 did not differ notably from the other patients as to total-platinum kinetics, but in Patient 10, who had renal impairment, the initial urinary platinum excretion was reduced. Therefore, further studies on free-platinum kinetics should be performed in patients with subnormal renal function, since the findings might indicate the extent to which cisplatin regimens should be adjusted for this group of patients.

It can be concluded that although NLIN has the best features for fitting concentration-time curves, details indicating phenomena such as an EHC may be smoothed away. Extensive sampling, as was done in our study, and the use of CSTRIP allowed an estimation of the EHC of cisplatin.

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Significance of the Low-Density Lipoprotein (LDL) Receptor Pathway for the In Vitro Accumulation of AD-32 Incorporated Into LDL in Normal and Leukemic White Blood Cells[1,2]

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The accumulation of the lipophilic cancer chemotherapeutic agent AD-32 as free drug and incorporated into low-density lipoprotein (LDL) (AD-32-LDL) has been studied in wbcs from normal subjects and from patients with acute myelogenous leukemia exhibiting high-affinity uptake and degradation of ^{125}I-LDL. The rate of degradation of ^{125}I-LDL in the leukemic cells was four to 25 times higher than that in normal wbcs. The accumulation of LDL-incorporated AD-32 was greater than that of the free drug and could be reduced by the addition of native LDL in excess. AD-32-LDL was as effective as native LDL in inhibiting the degradation of ^{125}I-LDL. Preincubation of wbcs in a lipoprotein-deficient medium enhanced both the degradation of ^{125}I-LDL and the cellular drug accumulation on incubation with AD-32-LDL. Heparin inhibited both the high-affinity degradation of ^{125}I-LDL and the cellular accumulation of LDL-incorporated drug. However, comparison of the high-affinity degradation rate of ^{125}I-LDL and the accumulation of LDL-incorporated AD-32 showed that the cellular drug accumulation markedly exceeded that which could be explained by high-affinity uptake and degradation of AD-32-LDL. Furthermore, saturation of the cellular uptake of LDL-incorporated drug occurred at higher LDL concentrations than saturation of ^{125}I-LDL degradation. The results indicate that cellular uptake of AD-32-LDL is partly mediated by the LDL receptor pathway and partly by a nonspecific mechanism. [Cancer Treat Rep 68:515–520, 1984]

The major problem in cancer chemotherapy is the lack of selectivity of the drugs used, which leads to severe toxic effects on normal cells. A possible way to increase the selectivity of a drug would be to link it to a specific carrier with high affinity for the malignant cells. Attempts have been made to administer cancer chemotherapeutic agents linked to antibodies (1), DNA (2), or liposomes (3) with, at least in some cases, enhanced toxic effects on tumor cells in vitro. On the other hand, the results of in vivo administration have been less encouraging because of immunologic reactions, rapid clearance, or stability problems with the drug-carrier complexes.

During the last few years, it has been shown that human cells express cell surface receptors for low-density lipoprotein (LDL), which is the major cholesterol-carrying lipoprotein in human plasma (4-6). Evidence has been presented that after binding to the receptor, LDL is internalized and degraded, presumably in lysosomes (5). The lipid core of LDL, containing about 1500 cholesteryl ester molecules, yields free cholesterol which is used for membrane synthesis, whereas the protein part of LDL (apoprotein B) is degraded to amino acids (4).

Recently, it has been shown that freshly isolated leukemic cells from patients with acute myelogenous leukemia (AML) have higher receptor activity than mononuclear cells and neutrophil granulocytes from healthy individuals (7,8). This raises the possibility that LDL particles can be used as endogenous drug carriers in the treatment of AML for targeting cancer chemotherapeutic agents to leukemic cells with high LDL receptor activity.

In the present study, we incorporated the lipophilic anthracycline derivative AD-32 into LDL particles. We then studied the mechanism for cellular accumulation of AD-32 incorporated into LDL (AD-32-LDL) in mononuclear

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cells from healthy individuals and in leukemic cells from AML patients.

MATERIALS AND METHODS

Materials

Sodium ^{125}iodide (carrier-free, pH 7–11) was obtained from the Radiochemical Centre, Amersham, UK; sodium metrizoate/Ficoll (Lymphoprep) from Nyegaard and Co, Oslo, Norway; and Sephadex gel filtration columns (PD 10) from Pharmacia Fine Chemicals, Uppsala, Sweden. AD-32 and Diluent 12 were gifts from Dr. Merwyn Israel, Dana-Farber Cancer Institute, Boston, MA. Falcon tissue culture dishes (35 × 10 mm) and sterile 50-ml Falcon plastic tubes were obtained from Becton Dickinson and Co, Oxnard, CA; tissue culture medium RPMI-1640 was obtained from Gibco-Biocult Ltd, Paisley, Scotland; and heparin (5000 IU/ml) was obtained from AB Kabi, Stockholm, Sweden.

Lipoproteins

Human LDL (density, 1.019–1.063 g/ml) was isolated by ultracentrifugation of serum from healthy individuals on a sodium chloride gradient (9). Human lipoprotein-deficient serum (LPDS) with a density of > 1.215 g/ml was prepared by ultracentrifugation of normal serum, after adjusting the density to 1.215 by addition of sodium bromide (10), and was then heat-inactivated by incubation at 56°C for 30 minutes. LDL was labeled with ^{125}I to a specific activity of 100–800 cpm/ng (11). Labeled LDL was passed through a Sephadex G 25 column (PD 10) and extensively dialyzed against 0.15 M NaCl containing 0.3 mM EDTA to remove free iodine, after which human serum albumin (200 mg/ml) was added to give a final concentration of 4% (wt/vol) and the preparation was filtered through a 0.45-μm Millipore filter (Millipore Corp, Bedford, MA). The purity of the LDL and LPDS was checked by agarose gel electrophoresis.

Incorporation of AD-32 Into LDL Particles

AD-32 was incorporated into native LDL using the method of Krieger et al (12) for incorporating hydrophobic compounds into LDL. In brief, aliquots containing 1.9 mg of LDL were lyophilized in the presence of potato starch and the neutral lipids were removed by extraction with heptane at -10°C. The heptane-extracted LDL was then reconstituted by the following sequential steps: 6 mg of AD-32 dissolved in diethyl ether was added, the solvent was evaporated under nitrogen and, finally, the reconstituted LDL was solubilized in 10 mM tricine (pH 8.4). As a control, AD-32 alone could not be dissolved in 10 mM tricine (pH 8.4) after evaporation of the solvent. The AD-32-LDL preparation that was obtained contained 0.7–1.4 mg of LDL protein and 0.35–1 mg of AD-32 (AD-32/LDL protein mass ratio = 0.5–0.7) and migrated as native LDL on agarose gel electrophoresis.

Isolation of Blood Cells

Human leukemic cells and normal mononuclear cells were isolated by centrifugation of heparinized peripheral blood on Lymphoprep (13). All normal cells were obtained from healthy blood donors at the Karolinska Hospital, Stockholm, Sweden. Clinical data on the patients with AML are given in the text. The cells studied contained 30–80 μg of cell protein/10^6 cells.

Cellular Degradation of ^{125}I-LDL

Freshly isolated, normal mononuclear and leukemic cells (3×10^6) were incubated with ^{125}I-LDL in 1 ml of RPMI-1640 supplemented with 30% LPDS. All incubations were performed in 35 × 10-mm tissue culture dishes at 37°C in humidified air containing 5% CO_2. The degradation of ^{125}I-LDL was determined from the amount of trichloroacetic acid-soluble radioactivity in the incubation medium after extraction of free iodine with chloroform (4). Blank incubations were performed under identical conditions in the absence of cells. High-affinity degradation was calculated by subtracting the amount of trichloroacetic acid-soluble radioactivity in the presence of a 20-fold excess of unlabeled LDL (nonspecific degradation) from the amount of ^{125}I-LDL degraded in the absence of unlabeled LDL (total degradation). This was used as a measure of the LDL receptor activity. In some experiments, the cells were preincubated for 60–70 hours in HEPES-buffered RPMI-1640 with 10% LPDS in sterile 50-ml Falcon plastic tubes on a rocker platform (Bellco Glass Inc, Vineland, NJ) to induce high LDL receptor activity, as described by Ho et al (14).

Drug Accumulation in White Blood Cells

The isolated cells were incubated with free AD-32 and AD-32-LDL in HEPES-buffered RPMI-1640 containing 10% LPDS in sterile plastic centrifuge tubes in a shaking water bath at 37°C. Free AD-32 was dissolved in Diluent 12, a mixture of ethanol and castor oil, and was diluted with saline as described by Israel et al (15). The incubations were performed in a volume of 1 ml at a cell concentration of 3×10^6 cells/ml and were terminated by the addition of 4 ml of ice-cold phosphate-buffered saline (PBS). Thereafter, the cells were centrifuged at 2000 rpm at 4°C for 5 minutes and washed twice with 4 ml of ice-cold PBS. The cells were then resuspended in PBS and kept at -20°C prior to analysis. Blanks refer to incubations under identical conditions but in the absence of cells.

Assays

AD-32 was assayed fluorometrically in a Shimadzu spectrofluorophotometer model RF-510 (Shimadzu Seisakusho Ltd, Kyoto, Japan) using 485 and 560 nm as excitation and emission wavelengths, respectively. The amount of AD-32 incorporated into LDL was determined after extraction of AD-32 with 12 vol of chloroform/methanol (4:1 vol/vol). The amount of AD-32 in cell samples was determined as total fluorescence after extraction as described above, following sonication of the cells with an MSE 100-watt ultrasonic disintegrator (catalog No. 7100, London, England). Protein was determined by the method of Lowry et al (16), using bovine serum albumin as a standard. Radioactivity was measured in a Packard autogamma scintillation spectrometer (Packard Instrument Co, Inc, Downers Grove, IL).

RESULTS

LDL Receptor Activities in Normal and Leukemic White Blood Cells

Figure 1a demonstrates that isolated mononuclear cells from a healthy subject degraded ^{125}I-LDL by a saturable, high-affinity process with a maximal velocity at about 30 μg of LDL protein/ml. Before the incubation with ^{125}I-LDL, the cells were preincubated in LPDS for 72 hours. The preincubation caused a tenfold increase in the high-affinity degradation rate, as compared to freshly isolated cells (data not shown). Figure 1b shows that freshly isolated leukemic cells degraded ^{125}I-LDL in a similar fashion.

Cellular Accumulation of AD-32

The time course for AD-32 accumulation in leukemic cells incubated with AD-32-LDL is shown in figure 2. The cells had been isolated from a 74-year-old female with AML who had a wbc count of 15 × 10^9/L. These cells exhibited a high-affinity degradation rate for ^{125}I-LDL that exceeded the values for freshly isolated normal mononuclear cells by about four times (data not shown). Cellular uptake was rapid during the first 20 minutes, followed by a slower phase. In the presence of a 20-fold excess of native LDL, there was a marked reduction in cellular drug uptake. Figure 3 shows the uptake of AD-32 in leukemic cells as a function of the drug concentration in the medium. These cells were obtained from a 55-year-old male with AML, who had a wbc count of 35 × 10^9/L. The high-affinity degradation rate for these cells was 25 times that of freshly isolated, normal mononuclear cells (data not shown). The drug uptake was considerably higher when the cells were incubated with AD-32-LDL than on incubation with the free drug. When the AD-32-LDL concentration was increased, drug uptake increased rapidly with the complex concentration up to an AD-32 concentration of about 40 μg/ml, corresponding to an LDL concentration of 66 μg/ml. Further increase in the complex concentration caused only a slight increase in the drug uptake.

Inhibition of ^{125}I-LDL Degradation of AD-32-LDL

The addition of equivalent amounts (as determined from the protein concentration) of AD-32-LDL and native LDL to the medium inhibited the degradation of ^{125}I-LDL to the same extent in normal mononuclear cells that had been preincubated in LPDS to induce a high LDL receptor activity (fig 4).

Comparison of LDL Receptor Activity and Accumulation of AD-32

The uptake of AD-32-LDL in normal mononuclear cells that had been preincubated in LPDS for 65 hours to induce a high LDL receptor activity was compared to the uptake in identical cells that had been preincubated in

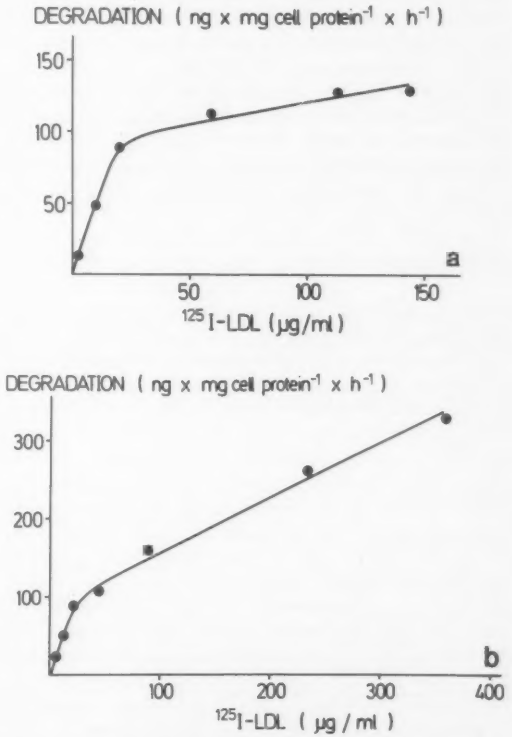

FIGURE 1.—Degradation of ^{125}I-LDL by normal mononuclear cells (a) and leukemic cells (b) as a function of ^{125}I-LDL concentration. The incubation time was 6 hrs. Prior to the incubation with ^{125}I-LDL, the normal mononuclear cells had been preincubated in a medium containing 10% LPDS for 72 hrs.

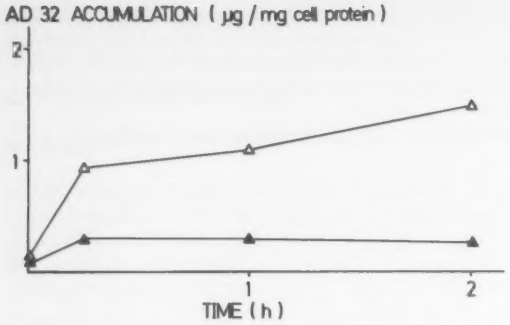

FIGURE 2.—Time course for the accumulation of AD-32 in leukemic cells in the absence (△) and presence (▲) of 500 µg/ml of native LDL. The incubation was carried out with AD-32-LDL at a concentration of 30 µg/ml, corresponding to 18 µg/ml of AD-32.

LPDS plus native LDL to prevent induction of LDL receptors. Figures 5a and 5b demonstrate that the cells with the highest LDL receptor activity also accumulated the most AD-32 on incubation with AD-32-LDL. In contrast, the induction of LDL receptor activity did not influence cellular drug accumulation during incubation with free AD-32. Incubation with AD-32-LDL at 4°C resulted in considerably less drug accumulation than incubation at 37°C. The addition of heparin to the incubation medium counteracted the enhanced drug uptake in cells preincubated with LPDS (fig 6a). Moreover, heparin also greatly reduced the high-affinity degradation of ^{125}I-LDL (fig 6b).

DISCUSSION

The present results show that LDL-incorporated AD-32 accumulates in freshly isolated leukemic cells, as well as

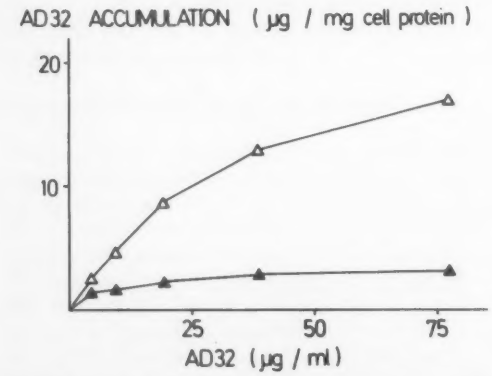

FIGURE 3.—Cellular accumulation of AD-32 as a function of the concentration of free AD-32 (▲) and AD-32 incorporated into LDL (△). The incubation time was 1 hr. 1 µg of AD-32 corresponded to 1.7 µg of LDL in AD-32-LDL.

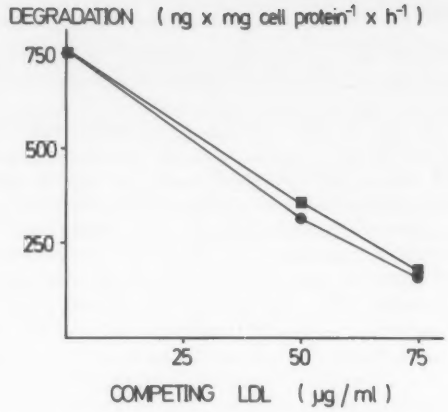

FIGURE 4.—Effect of AD-32-LDL (■) and native LDL (●) on the degradation of ^{125}I-LDL (25 µg/ml) by normal mononuclear cells. The incubation time was 4 hrs. Prior to the competition study, the cells had been preincubated in a medium containing 10% LPDS for 65 hrs, washed with medium, and re-counted.

in normal mononuclear cells. The accumulation was much higher than that of the free drug. In the future, AD-32 incorporated into LDL might prove to be an alternative way to administer AD-32. At present, free AD-32 is dissolved in a mixture of ethanol and castor oil, leading to lung complications and other allergic reactions (17).

AD-32-LDL was as effective as native LDL in inhibiting the degradation of ^{125}I-LDL, and the addition of native LDL in excess inhibited the accumulation of AD-32-

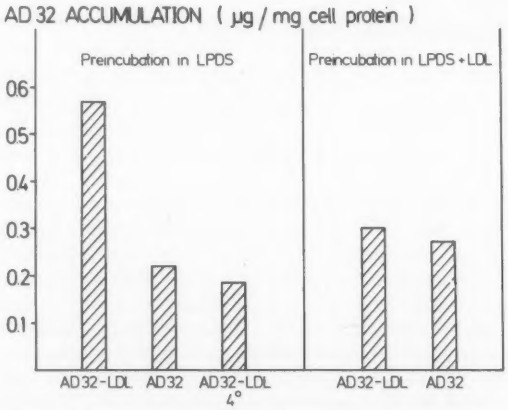

FIGURE 5a.—Accumulation of AD-32 in normal mononuclear cells on incubation with AD-32-LDL and free AD-32. The cells (2 × 10^6 cells/ml) were preincubated in a medium containing 10% LPDS in the presence and absence of 80 µg/ml of native LDL. After 65 hrs of preincubation, the cells were washed with medium, re-counted, and incubated with 18 µg/ml of free AD-32 or AD-32 incorporated into LDL for 1 hr and 15 mins (protein concentration of AD-32-LDL = 25 µg/ml).

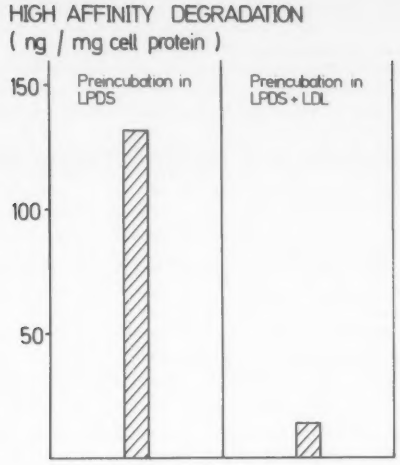

FIGURE 5b.—High-affinity degradation of ^{125}I-LDL in the cells used for AD-32 accumulation studies in fig 5a. The incubation time was 1 hr and 15 mins.

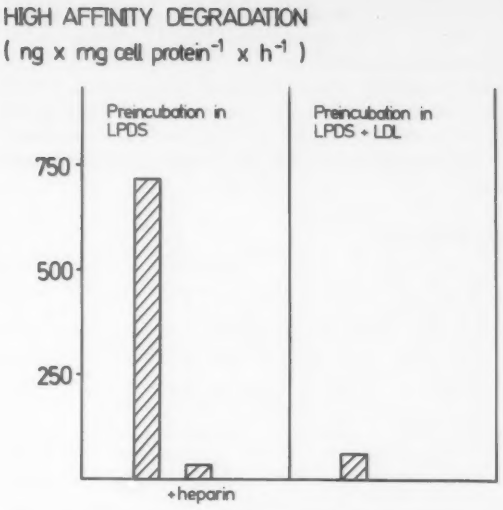

FIGURE 6b.—High-affinity degradation rate of ^{125}I-LDL for the cells studied in fig 6a. The incubation time with ^{125}I-LDL (25 μg/ml) was 4 hrs.

LDL, supporting a role for the LDL receptor in drug accumulation. Furthermore, induction of the LDL-receptor activity by preincubation of the cells in a lipoprotein-deficient medium enhanced the cellular accumulation of LDL-incorporated AD-32 but not that of free drug. Both the degradation of ^{125}I-LDL and the accumulation of LDL-incorporated drug were inhibited by heparin, and drug accumulation was also reduced by lowering the incubation temperature to 4 °C. Judging from the degradation of ^{125}I-LDL, saturation of the LDL receptors occurred at an incubation concentration of about 30 μg/ml. However, saturation of cellular accumulation of AD-32-LDL occurred at higher concentrations, indicating that

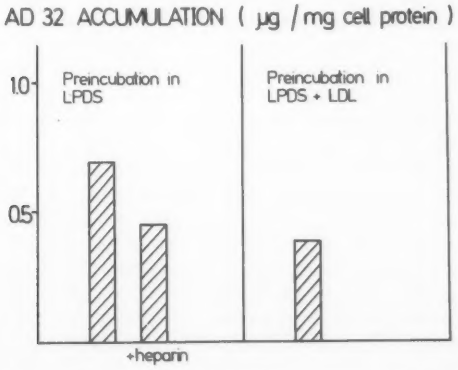

FIGURE 6a.—Effect of heparin (250 IU/ml) on the accumulation of AD-32 in normal mononuclear cells. The cells were preincubated as described in fig 5a. The incubation time with AD-32-LDL was 1 hr (protein concentration = 25 μg/ml, corresponding to 15 μg/ml of AD-32).

mechanisms other than LDL receptor-mediated drug uptake were involved. It has recently been found that LDL-associated benzo(a)pyrene accumulates in cultured fibroblasts by an LDL receptor-independent mechanism (18). The accumulation of LDL-incorporated AD-32 in leukemic cells occurred almost as rapidly as that of LDL-associated benzo(a)pyrene, which may indicate that this unknown mechanism also plays a role in the accumulation of AD-32 incorporated into LDL.

The current data do not allow one to exactly determine the contributions of receptor-dependent and non-receptor-dependent mechanisms to the total cellular uptake of LDL-incorporated AD-32. However, the data of figure 5b demonstrate that approximately 20 ng of LDL/mg of cell protein was degraded by the cells preincubated in LPDS + LDL. At the end of the incubation, 20 ng of LDL/mg of cell protein was found associated with the cells (bound and internalized) (data not shown). Thus, cellular uptake and degradation of LDL can account for a drug accumulation of 24 ng/mg of cell protein, since 1 ng of LDL corresponded to 0.6 ng of incorporated AD-32. However, the cellular drug concentration on incubation with AD-32-LDL was found to be several times higher. On the other hand, the difference in accumulation of AD-32 between the mononuclear cells preincubated in LPDS + LDL and LPDS was approximately 240 ng/mg of cell protein, and this corresponds fairly well to the eightfold increase in LDL receptor activity as calculated from the accumulation and degradation of ^{125}I-LDL, as described above. The mechanism of the uptake that is not receptor-mediated is not clear at present. One explanation for nonspecific

drug uptake may be the partition into cells of drug that has not been fully incorporated into LDL during the reconstitution procedure.

Since leukemic cells have higher LDL receptor activities than normal wbcs, the uptake of cytotoxic drugs in leukemic cells may be enhanced in the future treatment of AML by administering the drug incorporated into LDL.

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Probenecid Inhibition of Methotrexate Cytotoxicity in Mouse L1210 Leukemia Cells[1]

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The effect of probenecid on methotrexate cytotoxicity has been measured using mouse L1210 leukemia cells in vitro. Cytotoxic effects were measured using a soft agar cloning technique. Cell exposure to varying concentrations of methotrexate at fixed concentrations of probenecid resulted in increased cell survival when compared with exposure to methotrexate alone. Cell exposure to a fixed concentration of methotrexate and varying concentrations of probenecid showed this effect to be dependent on the concentration of probenecid. Intracellular methotrexate concentrations were unchanged by the presence of probenecid. However, there was an effect of probenecid on progression of cells through the cell cycle which would tend to decrease the number of cells susceptible to the cytotoxic effects of methotrexate. [Cancer Treat Rep 68:521–525, 1984]

Methotrexate is an effective chemotherapeutic agent against leukemia and other tumors (1–4). The drug is known to inhibit the enzyme dihydrofolate reductase (5) and thereby diminish the intracellular reduced folate pools necessary for DNA and protein synthesis (6). A number of in vitro studies have shown that the cytotoxicity of methotrexate exposure correlates with intracellular free-drug concentration and with duration of exposure (7). Based on these observations, attempts have been made to increase the concentration x time exposure of malignant cells to methotrexate by enhancing both systemic and central nervous system levels of drug and maintaining these levels for longer periods of time (8). One method of achieving this enhancement is to administer other drugs which decrease the clearance of methotrexate from plasma or cerebrospinal fluid. Several studies have shown that probenecid decreases methotrexate clearance from plasma and from cerebrospinal fluid, in animals (9–11) and in man (12), by inhibiting an active transport excretion process. While these studies demonstrated that probenecid increased both plasma and cerebrospinal fluid levels of methotrexate for protracted periods of time, the effect of probenecid on methotrexate cytotoxicity was uncertain. We therefore undertook the present study to examine the effect of probenecid on methotrexate cytotoxicity in mouse L1210 leukemia cells. The results indicate that, using cloning efficiency as a measure of cell survival, probenecid exposure diminishes the cytotoxic effect of methotrexate.

MATERIALS AND METHODS

Mouse L1210 leukemia cells were maintained in exponential growth phase in suspension culture, using RPMI-1630 medium supplemented with 20% fetal calf serum. Methotrexate was obtained from the Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The drug was dissolved in distilled water at a concentration of 0.1 M and was stored frozen. Probenecid was also obtained from the Division of Cancer Treatment, National Cancer Institute. Stock solutions were made up in distilled water at a concentration of 100 mg/ml and were stored frozen. ^{3}H-methotrexate (14.8 Ci/mmol) was obtained from Amersham Corporation, Arlington Heights, IL, and was used within 1 month of synthesis.

Six hours prior to drug exposure, aliquots of cells were resuspended in RPMI-1630 medium supplemented with 20% dialyzed fetal calf serum. Dialyzed fetal calf serum was prepared by three successive dialyses against 10 vol of phosphate-buffered saline at 4°C, as described by Pinedo et al (13). One hour prior to drug exposure, cells were resuspended in fresh medium using dialyzed fetal calf serum at a concentration of 4×10^5 cells/ml. Drugs were

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added 1 hour later from thawed stock solutions to yield appropriate final concentrations. After drug exposure, cells were washed twice with medium and then resuspended in fresh medium supplemented with dialyzed fetal calf serum. Cell survival was measured using colony-forming ability in soft agar, as described by Chu and Fischer (14). Cells treated as described above, but not exposed to drug, had a colony-forming efficiency of 80%. Intracellular uptake of methotrexate was measured using ^{3}H-methotrexate, as described by Goldman et al (15). Determinations were made at methotrexate concentrations of 10^{-6} and 10^{-5} M, with and without the presence of probenecid (275 μg/ml).

Cell cycle effects of probenecid were assessed using a modification of the flow microfluorometry technique previously described by Krishan (16). Cells were exposed to varying concentrations of probenecid for 24 hours. At the end of the exposure time aliquots containing 2×10^{6} cells were centrifuged and the pellet was resuspended in 0.25 ml of 0.1% sodium citrate solution containing propidium iodide (50 μg/ml). The mixture was allowed to stand for 10 minutes at room temperature, after which 0.25 ml of 1.12% sodium citrate solution containing RNAase[A] (500 units/ml) was added. This mixture was then allowed to stand at room temperature for 10 minutes. Relative cellular fluorescence was then measured using a FACS II cell sorter. The relative quantity of DNA in S phase was estimated graphically from the fluorescence curves.

RESULTS

When mouse L1210 leukemia cells were exposed to methotrexate continuously for 24 hours, cell survival, as measured by colony-forming ability in soft agar, decreased progressively with increasing methotrexate concentration up to a drug concentration of 10^{-5} M (fig 1). No further reduction in cell survival was observed at higher methotrexate concentrations. The addition of probenecid, at a concentration of 27.5 μg/ml, did not materially alter the concentration dependence of methotrexate cytotoxicity. The addition of a higher concentration of probenecid (275 μg/ml) markedly reduced methotrexate cytotoxicity of all concentrations. However, the relative insensitivity of cell survival to methotrexate concentrations $> 10^{-5}$ M was maintained, with measured cell survivals at concentrations $> 10^{-5}$ M methotrexate approximately equal to those observed at 10^{-5} M.

To define this effect of probenecid more clearly, cells were exposed to 10^{-4} M methotrexate for 24 hours in the presence of increasing concentrations of probenecid, (from 25 to 400 μg/ml). The results are shown in figure 2. If the cloning efficiency of cells exposed to 10^{-4} M methotrexate without probenecid added is taken as unity, the addition of increasing concentrations of probenecid increases the relative cloning efficiency of the cells (fig 2). This increase is dependent on probenecid concentration in a log linear manner, up to a probenecid concentration of 325 μg/ml, and less dependent on probenecid concentration > 325 μg/ml.

Colony-forming ability was unaffected by the presence of probenecid alone in the medium, up to a concentration of 425 μg/ml. However, the growth rate of the cells appears to be altered in the presence of probenecid. Figure 3 shows a series of growth curves for mouse L1210 leukemia cells in the presence of various concentrations of probenecid. As the concentration of probenecid increases, the growth rate, as measured by cell number, decreases. At a probenecid concentration of 400 μg/ml, almost no change in cell number is observed as a function of time.

The results of flow cytofluorometric measurements on cells exposed to varying concentrations of probenecid for 24 hours are shown in figure 4. As the concentration of probenecid increases the relative proportion of cells in S phase decreases from approximately 25% in samples containing no probenecid to < 3% in samples containing ⩾ 135 μg/ml of probenecid. At the highest concentrations of probenecid there is a relative increase in the propor-

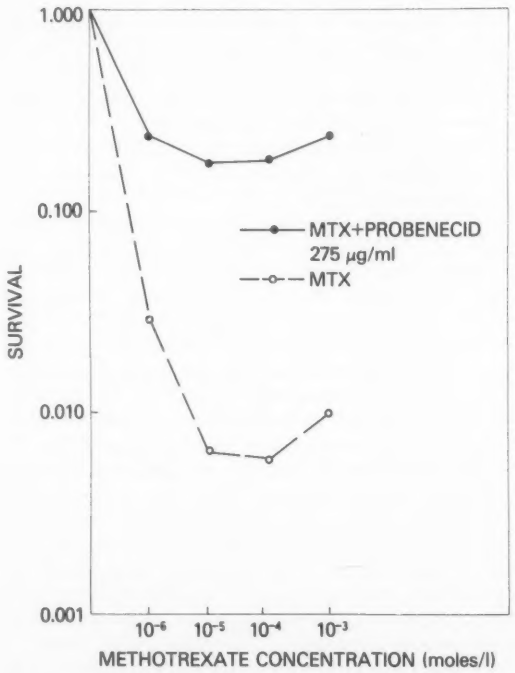

FIGURE 1.—Effect of probenecid on methotrexate cytotoxicity. Cells were incubated, as described in the Materials and Methods section, with varying concentrations of methotrexate in the presence (—●—) or absence (--○--) of probenecid (275 μg/ml) in the medium for 24 hrs. Cell survival was measured by cloning efficiency in soft agar.

tion of cells in G2 as well as an increase in the pre-G1 peak, which presumably represents cellular debris from nonviable cells.

We also measured uptake of radiolabeled (^{3}H) methotrexate into mouse L1210 leukemia cells, after exposures of 20 minutes in 10^{-6} M methotrexate, with and without probenecid (275 μg/ml) present, using the method of Goldman et al (15). No difference in cellular incorporation of radiolabeled compound was noted (1866 cpm/mg dry weight in the absence of probenecid and 1933 cpm/mg dry weight in the presence of probenecid).

DISCUSSION

The data outlined above indicate that probenecid has a concentration-dependent inhibitory effect on the cytotoxicity of methotrexate. The mechanism by which this effect occurs is uncertain, but we have explored some possibilities. Probenecid is known to inhibit active transport of methotrexate out of cerebrospinal fluid (11, 12) and to inhibit renal clearance of the drug from plasma (9). Probenecid therefore might inhibit cellular uptake of methotrexate as well. The shape of the methotrexate cytotoxicity curve in the presence of probenecid, when compared with the same curve in the absence of probenecid (fig 1), does not support probenecid inhibition of methotrexate transport as a mechanism for reduced cytotoxicity. One would expect that increasing concentrations of methotrexate, in the presence of probenecid, would result in increasing cytotoxicity, if inhibition of transport were a major component of the effect, particularly at methotrexate concentrations > 10^{-6} M, where diffusion processes play a more prominent role in methotrexate ingress into cells. Instead, the curve in the presence of probenecid is similar in shape to that observed without probenecid in the medium, with little increase in cytotoxicity noted between 10^{-6} and 10^{-3} M methotrexate, a finding which is not consistent with probenecid inhibition of methotrexate transport into the cells as a mechanism for the decrease in cytotoxic effect. However, no difference in ^{3}H-methotrexate uptake was observed with or without probenecid (275 μg/ml) present.

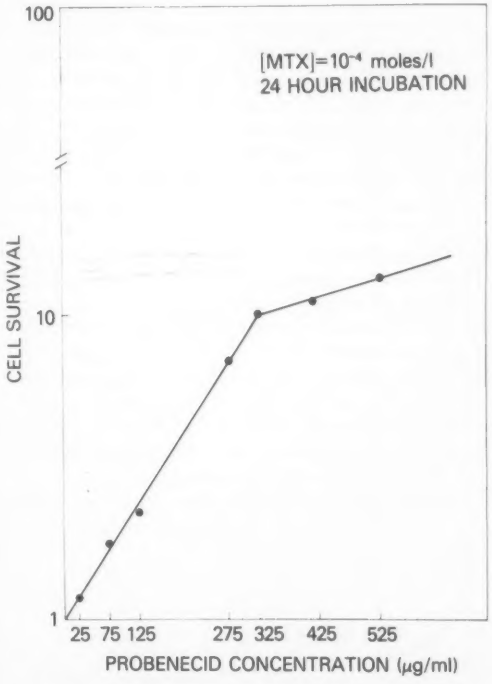

FIGURE 2.—Dependence of methotrexate cytotoxicity on probenecid concentrations. Cells were incubated in medium containing a defined concentration of methotrexate (10^{-4} M) and varying concentrations of probenecid for 24 hrs. Cell survival was measured by cloning efficiency in soft agar. Cell survival is shown as a function of probenecid concentration in the medium, relative to the survival obtained in the absence of probenecid (but in the presence of 10^{-4} M methotrexate).

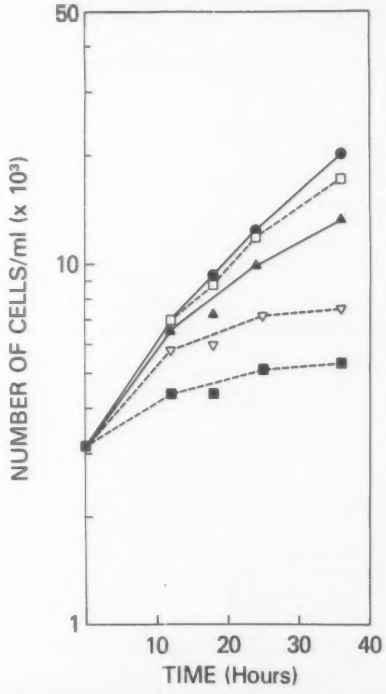

FIGURE 3.—Effect of probenecid on cell growth. Cells were grown in medium, as described in the Materials and Methods section, to which various concentrations of probenecid were added. Cell No. (per ml) was then determined as a function of time. Concentrations of probenecid in the medium (μg/ml) were 0 (●), 50 (□), 100 (□, same curve), 200 (▲), 400 (▽), and 800 (■). The growth rate decreases with increasing probenecid concentration.

Another possible mechanism for the effect of probenecid could relate to its effect on dihydrofolate reductase, the principal enzyme inhibited by methotrexate exposure. Measurements of dihydrofolate reductase activity in the presence and absence of probenecid showed no change, and the effect of methotrexate on dihydrofolate reductase activity appeared unaltered by the presence of probenecid.

The alteration in the methotrexate cytotoxicity curve in the presence of probenecid (fig 1) suggests that the effect of probenecid is seen at all concentrations of methotrexate. The data in figure 2 suggest that the effect is dependent on the concentration of probenecid in the medium. Another possible mechanism of probenecid effect would therefore be an alteration in cellular susceptibility to methotrexate that was dependent only on probenecid concentration. Since methotrexate exerts its cytotoxic effect on dividing cells, predominantly in S phase, an alteration in the kinetics of growth of the cells and a consequent alteration in the fraction of the cell population susceptible to the cytotoxic effect of methotrexate might play a significant role in the protective effect of probenecid. Results shown in figure 3 demonstrate a dose-dependent inhibition of cell growth by probenecid, supporting this as a factor in the probenecid effect.

The results shown in figure 4 are also consistent with this idea. Flow microfluorometric measurements on cells exposed to varying probenecid concentrations indicate minimal change at low probenecid concentrations but decreasing fractions of cells in S phase at higher probenecid concentrations. At these higher concentrations, increasing numbers of cells are in the G2 phase, and an increasing amount of cellular debris is also noted. Other data obtained during these studies indicate that the cloning efficiency of the cells studied is approximately 80% and is independent of probenecid concentration (up to 400 μg/ml). The net results of these findings are compatible with the results shown in figure 3, which indicate that the rate of increase in cell number decreases with increasing probenecid concentration. They are also compat-

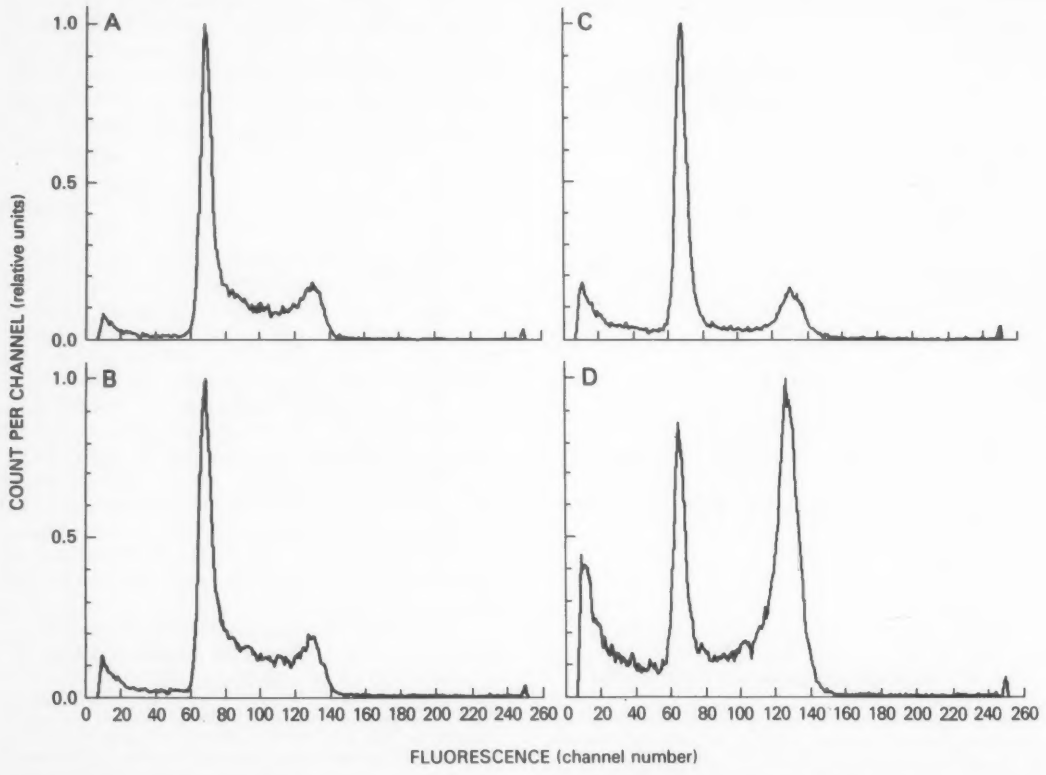

FIGURE 4.—Flow cytofluorometric analysis of L1210 murine leukemia cells after exposure to probenecid in vitro for 24 hrs. A number of concentrations were used: 0 (panel A), 27 μg/ml (panel B), 135 μg/ml (panel C), and 275 μg/ml (panel D). With increasing probenecid concentration, there is a relative decrease in S phase and increases in the G2 phase and in material seen "pre G1," which is probably cellular debris.

ible with the results of figure 2, in that a decreasing fraction of cells are in S phase, and sensitive to methotrexate, with increasing probenecid concentration.

The overall results of these studies suggest that exposure of tumor cells to methotrexate in the presence of high concentrations of probenecid is associated with reduced cytotoxicity, compared with exposure to methotrexate alone.

A number of studies have indicated that probenecid given in vivo enhances methotrexate exposure by decreasing renal and hepatic clearance rates for methotrexate. The relative effects in vivo of decreasing clearance and potentially enhancing methotrexate cytoxicity, and the decreased cytotoxicity of methotrexate at a cellular level in the presence of probenecid which we have shown here, are dependent on the balance between these effects. This balance varies with probenecid concentration. Methotrexate toxicity in animals has been shown to be increased by concomitant administration of probenecid (17). The administration of probenecid decreased both hepatic and renal clearance of methotrexate and resulted in higher plasma methotrexate levels. An explanation of the increased toxicity noted in this study may be, in part, related to the findings of a subsequent in vitro study by Sirotnak et al (18), in which the authors demonstrated that, for murine leukemia cells (L1210), there is a differential effect of probenecid on the influx and efflux of methotrexate. At lower concentrations of probenecid, efflux is predominantly affected, with resulting increased intracellular accumulation of methotrexate and increased cytotoxicity. At higher concentrations of probenecid, influx is reduced and methotrexate cytotoxicity potentially reduced. In the lower ranges of clinically useful doses of probenecid, relatively low plasma concentrations are achieved (19). The predominant effect on methotrexate pharmacokinetics is therefore to decrease clearance and to increase plasma levels. Cellular cytotoxicity is therefore increased. If higher levels of probenecid concentrations are achieved, the present work indicates that the balance would shift so that the cytotoxic effect of methotrexate would be reduced. This reduction may counteract the effect of the enhanced levels and may result in a less clinically useful antitumor effect when compared with methotrexate given alone.

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Aclarubicin-Associated QT_c Prolongation and Ventricular Fibrillation[1,2]

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A marked prolongation of QT_c (corrected QT interval for heart rate) in ECG developed in two patients with acute leukemia during induction chemotherapy with aclarubicin, one of the anthracycline antibiotics. This ECG change was followed by ventricular fibrillation. No signs or symptoms of congestive heart failure were noted. The outcome in one case was fatal; in the other case, the ECG change was reversible. These observations suggest that QT_c prolongation, which has been considered to be a sign of acute cardiotoxicity of anthracycline derivatives, may signal a clinical risk of ventricular fibrillation. [Cancer Treat Rep 68:527–529, 1984]

The anthracycline derivatives are widely used in the management of acute leukemia and various types of solid tumors. Unfortunately, they are associated with well-known cardiotoxicity, which limits their prolonged use. Aclarubicin (ACM), which is isolated from *Streptomyces galilaecus* (1), has been reported to have significantly reduced cardiotoxicity as compared with either daunorubicin or doxorubicin at equivalent therapeutic doses in animals (2). In the ECG for two patients with acute leukemia, a marked prolongation of QT_c (corrected QT interval for heart rate), which was followed by ventricular fibrillation without accompanying congestive heart failure, was observed during induction chemotherapy with ACM. As this type of acute cardiotoxicity has not yet been noted in antileukemic treatment with ACM, the clinical course of these two patients will be reported here in detail.

CASE REPORTS

Patient 1

A 44-year-old male was admitted to the hospital with a diagnosis of erythroleukemia. He had no past medical history concerning the cardiovascular system. His chest x-ray and ECG were unremarkable. On the 67th day of hospitalization, combination chemotherapy with daily bolus iv of ACM, iv infusion of N^4-behenoyl-β-arabinofuranosylcytosine (BH-AC), oral 6-mercaptopurine (6-MP), and oral prednisolone was started. In this regimen (BH-AC AMP), the daily dose of ACM was 20 mg. On the tenth day of treatment, QT_c was lengthened from 0.425s to 0.459s. Serum Ca level was 8.4 mg/dl, corrected Ca was 9.1 mg/dl, and K was 4.1 mEq/L. On the 12th day of treatment, the patient's body temperature rose to 38°C and watery diarrhea developed. On the 14th day of treatment, he complained of palpitation, even at bed rest, while high fever and diarrhea persisted. ECG recorded at that time showed transient atrial fibrillation with QT_c prolongation. Serum Ca was 7.9 mg/dl, corrected Ca was 8.9 mg/dl, and K was 3.7 mEq/L. A chest radiograph revealed neither cardiomegaly nor increased vascular markings. The BH-AC AMP regimen was discontinued. Atrial fibrillation subsided after digitalis administration was started. However, the patient continued to feel palpitation, and QT_c prolongation persisted. On the eighth day from the last ACM administration, serum K and Ca decreased to 2.7 mEq/L and 7.0 mg/dl, respectively. The corrected Ca level was 8.4 mg/dl. On the tenth day from the last administration of ACM, ventricular fibrillation developed suddenly and was followed by cardiac arrest within several minutes. The patient did not respond to cardiopulmonary resuscitation. Autopsy was not permitted.

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Patient 2

A 17-year-old female with a diagnosis of acute myelogenous leukemia was admitted to the hospital. Over the next 7 months, she received six courses of combination chemotherapy with DCMP (daunorubicin, cytarabine, 6-MP, and prednisolone) and one course of BH-AC plus doxorubicin, 6-MP, and prednisolone as induction chemotherapy; total doses of 480 mg/m^2 of daunorubicin and 107 mg/m^2 of doxorubicin were administered. Although a small number of leukemic blasts were persistently observed in her bone marrow, the patient was fully active and asymptomatic. Chest radiographs and ECG were within normal limits. One month after the last course of this combination chemotherapy, treatment with ACM was started at a daily dose of 20 mg as a drip infusion over 3 hours in order to achieve complete remission. On the 16th day of treatment, a tonic convulsion and syncopal attack developed suddenly and subsided spontaneously within a few minutes. A chest radiograph was unremarkable and there were no clinical signs suggesting cardiac failure. An ECG taken immediately after the attack revealed QT_c prolongation (0.540s) and ST-T changes. Electrical alternans was also observed. The serum Ca level was 9.4 mg/dl, corrected Ca was 8.9 mg/dl, and K was 4.4 mEq/L. ACM was discontinued. On the next day, a similar convulsion and syncopal attack developed again and an ECG taken at attack revealed ventricular fibrillation. Infusion of lidocaine was started to maintain sinus rhythm. Although QT_c prolongation persisted, no arrhythmia developed after discontinuation of lidocaine infusion 2 days later. Thereafter, the patient's clinical condition was uneventful with supportive therapy. Her leukemia relapsed 4 months later and she died of cerebral bleeding. Autopsy findings by light microscopy demonstrated leukemic cell infiltration in the endocardium and myocardium of her heart. Vacuolization and atrophy of myocardial cells were noted on some parts of the myocardium. However, no damage to the conduction system was observed.

DISCUSSION

In Patient 1, QT_c prolongation had already been observed before the appearance of electrolyte disturbance. Therefore, it remains very likely that ACM-associated processes may have accounted for QT_c prolongation. However, the total cardiac effect of hypokalemia, hypocalcemia, and ACM may have triggered ventricular fibrillation.

Patient 2 was clinically stable and had no electrolyte disturbance up to the time of the syncopal attacks. Therefore, it seems highly plausible that ACM played an important role in the development of QT_c prolongation followed by ventricular fibrillation. As a total dose of 586 mg/m^2 of anthracycline had already been delivered prior to administration of ACM, pre-existing cardiomyopathy due to prior anthracycline administration might be conjectured.

In these patients, QT_c prolongation followed by ventricular fibrillation developed when cumulative ACM doses of 280 and 320 mg had been administered for a duration of 14 and 16 days, respectively. These doses are < 550 mg/m^2, which is the maximum dose of doxorubicin permitted for safe utilization (3). Therefore, it seems likely that acute rather than chronic cardiotoxicity should be suspected with these ECG abnormalities.

Prolonged QT interval has been considered to be a transient and reversible ECG abnormality for acute cardiotoxicity (4). However, our experience in these two cases seems to suggest that QT_c prolongation, in some particular situations, may lead to ventricular fibrillation, which is an extremely rare ECG abnormality of acute cardiotoxicity. To our best knowledge, only two cases have been reported in which ventricular fibrillation developed without symptoms of cardiac failure, when total amounts of 160 mg/m^2 (5) and 90 mg/m^2 (6) of daunorubicin had been administered.

The mechanism of QT_c prolongation due to ACM was unknown. It is well-known that quinidine causes prolonged QT interval. This drug decreases the rate of depolarization, prolongs the cardiac action potential, and delays repolarization. Weiss et al (7) very recently reported QT prolongation due to amsacrine, a new acridine derivative. It remains uncertain whether or not ACM and amsacrine have a cardiac effect similar to that of quinidine.

Although ACM has been reported to have only a mild degree of cardiac toxicity in phase I–II clinical studies (8,9), a significant increase in the incidence of arrhythmias was noted recently by continuous monitoring of ECG (10). Among these ECG changes, one patient showed a high degree of atrioventricular block. This case and our two cases suggest that ACM has the potential to cause or contribute to the development of serious cardiac arrhythmias. Further investigations are required for an understanding of the cardiotoxic potential of ACM.

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Phase I Study of Continuous-Infusion PALA and 5-FU[1]

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Eighteen patients with malignancies refractory to conventional forms of therapy were treated with 5-day continuous infusions of PALA and 5-FU. PALA was administered at a dose of 940 mg/m²/day. 5-FU was initially given at a dose of 180 mg/m²/day and was incrementally increased to 325 mg/m²/day. The courses were repeated every 3–4 weeks. Mucositis and diarrhea were the dose-limiting toxic effects. Other reversible side effects included grade 2 skin rashes and nausea and vomiting. Peak plasma PALA concentrations were approximately 20 μM and occurred in conjunction with a maximum depression of leukocyte-L-aspartate transcarbamylase (ATCase) activity to 10% of baseline. Therapeutic responses occurred in one of the 13 patients with colon carcinoma and in one patient with mammary carcinoma. Responses could not be correlated with leukocyte ATCase depression. Recent data indicate that low doses of PALA (250 mg/m²) might be equally as effective in inhibiting leukocyte ATCase activity as the doses used in this study. A phase II trial has been designed at this institution employing the above doses of PALA in conjunction with escalating doses of 5-FU. [Cancer Treat Rep 68:531–534, 1984]

A synergistic antitumor effect has been demonstrated for the combination of PALA and 5-FU in a human mammary carcinoma (MDA) cell line in culture (1), $CD8F_1$ mammary carcinoma (2), colon 38 and 26 carcinomas, Lewis lung carcinoma, and M5076 carcinoma (3). The apparent biochemical basis for this synergism involves an enhancement by PALA of the ratio of 5-FUTP to the total 5-FUTP and UTP pool, resulting in an increased incorporation of FUTP into RNA over that seen when 5-FU is used alone (1,4). Earlier clinical studies using bolus injections of PALA and 5-FU in combination showed no significant enhancement of 5-FU activity by PALA (5–10).

In vitro data have indicated that continuous prolonged exposure to 5-FU is more cytotoxic to human epithelial and colon carcinoma cells in vitro than intermittent exposure (11,12). Based on these findings, this phase I study was designed to evaluate the toxicity of PALA and 5-FU administered by 5-day simultaneous continuous infusions.

PATIENTS AND METHODS

PALA was administered at a dose of 940 mg/m²/day, which is approximately 60% of the maximum tolerated dose of PALA when administered as a single agent on a daily × 5 schedule (13–15). The dose of 5-FU was initially 180 mg/m²/day and was incrementally increased to 325 mg/m²/day. The drugs were given on a schedule of every 3–4 weeks. Dose escalation in new patients was carried out after a minimum of three patients had completed cycles of therapy at the previous dose level. Dose escalations within an individual patient were allowed, providing the patient had completed the course at the prior dose level with no toxicity. The pretreatment characteristics are shown in table 1.

RESULTS

Toxicity

Eighteen patients completed 49 courses of treatment. Side effects were similar to those seen in previous studies (5–10) and included mucositis, diarrhea, skin rashes, and nausea and vomiting (table 2).

In this study, mucositis varied from angular stomatitis to complete oral mucosal ulceration, most commonly started on the 7th to 8th day of treatment, and persisted for approximately 7 days. Four patients required treat-

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TABLE 1.—Characteristics and toxic effects of patients treated with continuous-infusion PALA and 5-FU*

	No. of patients
Total evaluable	18
Sex	
Male/female ratio: 1:2	
Age in yrs	
Median: 60	
Range: 21-76	
Performance status (Karnofsky)	
≥ 60	16
40-60	2
Prior therapy	
Chemotherapy	17
Radiation therapy	7
Primary tumor†	
Colorectal carcinoma	13
Fibrosarcoma	1
Mammary carcinoma	1
Pancreatic carcinoma	1
Endometrial carcinoma	1
Leiomyosarcoma	1

*5-FU and PALA were diluted separately in 500 ml of 0.45% sodium chloride and were given as 24-hr continuous infusions for 5 days. Initially, the drugs were administered at separate iv sites. In the latter part of the study, however, both drugs were given through the same iv line for patient convenience.

†Sites of metastasis: lung (11 patients), liver (11), bone (2), and pelvis (1).

ment with cimetidine or Mylanta. One patient treated at a dose level of 325 mg/m^2 of 5-FU became temporarily hypovolemic secondary to fluid loss due to vomiting. Generalized skin rashes were also observed but showed no relationship to dosage level. Patients most commonly experienced a mild to moderate occasionally pruritic maculopapular desquamating eruption of the trunk, upper chest, and face, although one patient experienced a severe generalized dermatitis which lasted approximately 1 week at the 250-mg/m^2 dose level of 5-FU. Rashes were first observed on Day 9. In the two cases in which severe toxicity was observed (250 mg/m^2 of 5-FU), symptoms responded to therapy with prednisone. Local erythema and phlebitis occurred in patients who received PALA and 5-FU through the same iv line, but were not observed when the drugs were administered at separate sites. Neither myelosuppression nor neurotoxicity was observed.

Pharmacology and Antitumor Activity

In four patients, plasma was analyzed for PALA concentration and for leukocyte-L-aspartate transcarbamylase (ATCase), the enzyme specifically inhibited by PALA (16). Within 24 hours following the start of drug infusion, PALA plasma concentration had reached a steady-state plateau of approximately 20 μM (fig 1). During this same time interval, leukocyte ATCase activity had decreased to 10% of baseline values and remained at this level throughout the 96 hours of follow-up. Two patients who had previously received 5-FU achieved partial responses. A 68-year-old woman with colon carcinoma and documented metastatic disease in the lung who had previously received 5-FU and semustine experienced a partial regression of lung metastases (duration, 6 months) following two courses of therapy. A 44-year-old woman who had received cyclophosphamide, doxorubicin, and 5-FU chemotherapy for advanced metastatic breast carcinoma had a decrease in size of axillary lymph nodes (duration, 1 month) following two courses of therapy. Twelve patients had less than partial objective responses (1-6 months).

DISCUSSION

A combination of PALA (940 mg/m^2) and 5-FU (250 mg/m^2) given as continuous 5-day infusions is well-tolerated and could be safely used in phase II studies. These

TABLE 2.—Toxic effects

Toxic effect*	5-FU dose (mg/m^2)†		
	180	250	325
Nausea and vomiting			
Mild to moderate	4 (44)	9 (56)	2 (50)
Severe	—	—	1 (25)
Mucositis			
Mild to moderate	6 (66)	7 (43.8)	1 (25)
Severe	—	2 (12.5)	1 (25)
Diarrhea			
Mild to moderate	6 (66)	6 (37.5)	1 (25)
Severe	—	—	—
Skin			
Mild to moderate	3 (33)	2 (12.5)	1 (25)
Severe	—	2 (12.5)	—

*Mild to moderate = 1+ or 2+; severe = 3+.

†Values in table = No. of patients (% of total).

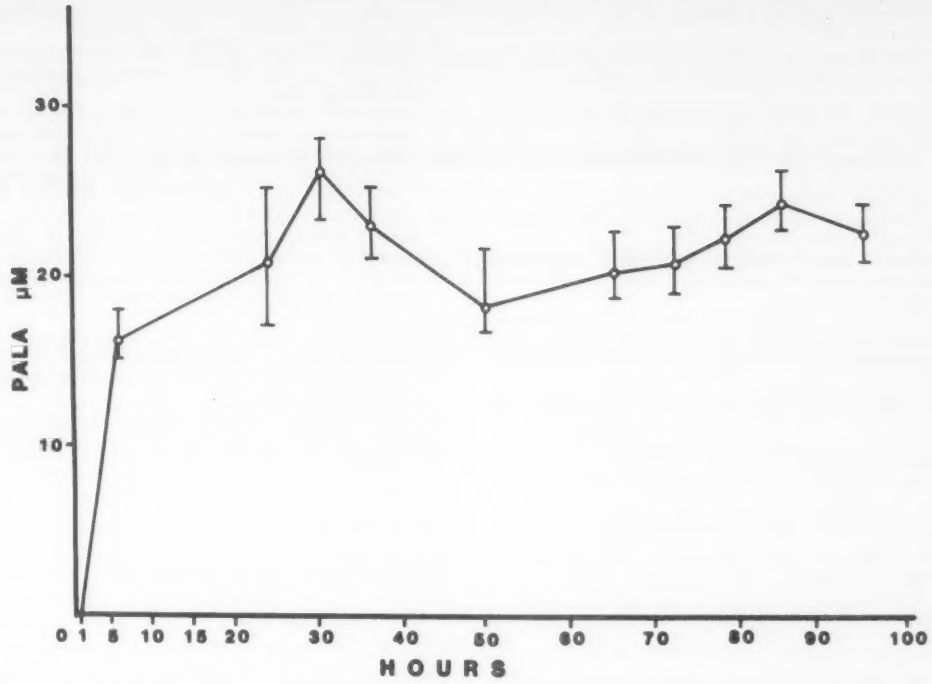

FIGURE 1.—Graph indicates plasma PALA concentration in 4 patients (mean ± SD). Dose of 940 mg/m^2 of PALA was given by continuous infusion daily × 5. Plasma concentration reached a steady-state plateau phase of approximately 20 µM, 24 hrs following the start of PALA infusion.

doses produce a sustained depression of ATCase activity. However, depression of ATCase activity may not necessarily be predictive of response, since none of the four patients who demonstrated a decrease in ATCase activity responded to therapy.

In theory, the reduction of ATCase activity should result in a decrease in cellular UTP and a consequent increase in the incorporation of 5-FUTP into RNA, producing enhanced tumoricidal activity. In this study, nucleotide triphosphate pools were not quantitated in either tumor or normal tissue, so a direct measure of PALA effect was not available.

Recent laboratory data have indicated that doses of PALA lower than those required for therapeutic activity can produce a significant depression in UTP pools in murine breast tumor (BALB/c × DBA/8)F_1 (17). In investigations which we have conducted in dogs, preliminary results demonstrate that a dose of 250 mg/m^2 given by iv infusion over 5 days is sufficient to interrupt the de novo synthesis of UTP by > 80%. Increasing the PALA dose to 1000 mg/m^2 suppresses UTP formation by only an additional 10%. It appears, therefore, that a much lower dose of PALA than was used in this and other clinical studies would be equally as effective in inhibiting UTP formation and would enable a higher dose of 5-FU to be administered without incurring excessive toxicity. Other investigators have found that PALA (850 mg/m^2) and 5-FU (300–630 mg/m^2) given by 5-day continuous infusions are well-tolerated, although minimally active (18).

At this institution, a phase II study has been designed utilizing PALA (250 mg/m^2) and escalating doses of 5-FU on a weekly schedule. The most recent dosage level of 5-FU has been 1300 mg/m^2; the maximum tolerated dose has not yet been reached.

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Phase I-II Trial of Anthracenedicarboxaldehyde in Patients With Refractory Adult Acute Leukemia[1,2]

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Anthracenedicarboxaldehyde (ADC), one of a series of anthracenedicarboxaldehyde hydrazones, was found in preclinical trials to have antitumor activity similar to that of doxorubicin and to be active against P388 and L1210 stem cell leukemias (1). Its mechanism of action may involve DNA intercalation and/or direct DNA binding (2). In vitro, ADC inhibits thymidine uptake into DNA four times more potently than doxorubicin (3). Animal toxicology studies have shown no cardiac toxicity when compared to doxorubicin (4). In clinical trials, leukopenia was found to be the dose-limiting toxic effect of ADC (3,5-7). Using the solid tumor maximal tolerated dose for leukopenia as the starting dosage level, a phase I-II trial was initiated to assess antileukemic and toxic effects of ADC in adult patients with relapsed leukemia.

MATERIALS AND METHODS

A total of 13 patients with relapsed leukemia were entered into this study. There were nine male and four female patients. Acute nonlymphocytic leukemia (ANLL) was diagnosed in 12 patients, and acute lymphoblastic leukemia (ALL) was diagnosed in one. The median age was 36 years (range, 29-71). The median Karnofsky performance status was 70% (range, 30%-90%). Four patients had achieved two or more prior complete responses (CR). Five patients had achieved one prior CR and four had never achieved a CR. Ten patients had received prior anthracyclines.

All patients had a bone marrow biopsy or aspirate which confirmed the diagnosis of relapsed ANLL or ALL. Criteria for entry also required recovery from previous treatment, bilirubin < 1.5 mg/100 ml, and creatinine < 1.5 mg/dl.

During induction, all patients were examined and had cbcs and platelet counts daily. Hepatic, renal, and other serum biochemical tests were performed weekly or as the clinical course indicated. Bone marrow aspirations were performed on all patients on entry in the study and subsequently at weekly intervals.

Courses of therapy were continued until patients achieved remission, developed unacceptable toxicity, or had disease progression. CR was defined as a normocellular marrow with normal erythroid and myeloid elements; with myeloblasts, promyelocytes, and other leukemic cells totaling $< 5\%$; and with normal peripheral blood platelet and wbc counts. Partial response (PR) was defined as $> 5\%$ but $< 25\%$ myeloblasts, with an otherwise normal marrow. Patients dying before completion of 2 weeks of therapy were considered to have had early deaths but were still considered evaluable for nonhematologic toxic effects.

In 11 patients, ADC was given as a 2-hour iv infusion daily for 3 days at four dose levels: 100, 200, 300, and 400 mg/m^2/day. The drug was diluted in 500 ml of 5% dextrose and infused into a free-running solution of 5% dextrose infusing into a large vein. Nine patients received one course of ADC. Two patients received two courses of ADC 3 weeks apart at dose levels of 100 and 200 mg/m^2/day. Two other patients each received two courses of ADC daily for 5 days at dose levels of 200 and 250 mg/m^2/day. All courses of ADC were administered in the hospital and vital signs were taken every 15 minutes during infusion and every 30 minutes for 2 hours after infusion.

RESULTS

One patient died of complications related to leukemia on Day 2 of therapy and was inevaluable for response. Marrow hypoplasia of < 2 weeks' duration occurred in four patients. Hypocellular marrow was not achieved in

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TABLE 1.—No. of courses in which toxicity occurred

Toxic effect	Total dose of ADC (mg/m^2)						
	3 days					5 days	
	200*	300	600	900	1200	1000	1250
Nausea/vomiting	0	0	1	3	1	2	2
Diarrhea	0	0	0	1	1	0	0
Mucositis	0	0	1	1	3	2	2
Phlebitis	0	1	1	0	0	0	0
Urticaria	0	0	0	0	1	0	0
Hypotension	0	0	1	2	1	0	1
Hepatic	0	1	1	3	2	0	1
Renal	0	1	0	2	2	0	0
Cardiac	0	0	0	2	2	0	0
No. of courses of ADC/total dose	1	2	4	3	3	2	2

* Patient death on Day 2.

the other eight patients. ADC caused a reduction in wbc count from a median count of 24,400/μl to a median nadir of 400/μl after a median of 9 days. Platelet transfusions were required by all patients.

Toxicity was significant and may have related to an increase in the total dose of ADC given (table 1). Toxicity was graded by Cancer and Leukemia Group B toxicity ratings. For 17 courses of ADC administered, grade 1-3 nausea and vomiting occurred in nine courses (53%), grade 2 diarrhea occurred in two (12%), grade 1-3 mucositis occurred in nine (53%), grade 2 urticaria occurred in one (6%), and grade 1-3 phlebitis occurred in two (12%). The low incidence of phlebitis in this study compared to other studies (5,6) may be explained by the administration of ADC over a longer time period through Hickman catheters which hastened drug dilution.

Hypotension defined as a decline of > 20 mm Hg in systolic blood pressure to a level < 90 mm Hg developed in five patients. The blood pressure reduction occurred 45-180 minutes after the start of ADC, lasted 30-75 minutes, and resolved without specific therapy. In four patients, hypotension occurred once during the 3 days of ADC. In the fifth patient, hypotension occurred on all 3 days.

Elevations in total bilirubin were observed in eight patients beginning 2-8 days after starting ADC and remained elevated for up to 21 days. The highest level recorded was 8.6 mg/dl in two patients on Days 10 and 13. Hepatic transaminase and alkaline phosphatase levels were unchanged by ADC.

In five patients, nonoliguric renal failure began 3-8 days after starting ADC. Creatinine and BUN levels peaked between Days 6 and 12. In three surviving patients, the median creatinine rise was 2.7 mg/dl and lasted between 9 and 39 days. Two patients were additionally receiving aminoglycoside antibiotics. Two other patients died with creatinine levels of 4.9 and 10.4 mg/dl, the first of whom was dialyzed. Neither patient had received aminoglycoside antibiotics.

Cardiac complications included congestive heart failure (CHF) in four patients, occurring between Days 5 and 9. Three of these patients had concurrent renal failure which may have contributed to the development of CHF. One of these patients also developed atrial arrythmias during her first dose of ADC.

DISCUSSION

In this study, antileukemic activity could not be determined because of too few patients at each dose level. However, marrow hypoplasia was not achieved in 75% of patients, which would suggest an increase in dose is necessary. With the treatment schedule used here, ADC appeared to cause several dose-limiting toxic effects. It is possible that all toxic effects were not solely drug related, since one side effect (renal failure) may have caused other medical abnormalities (CHF). Although cardiac toxicity has not been noted by others (3,5-7), the cardiotoxic potential of anthracenes at higher cumulative doses remains unknown. Further trials are still needed to assess antileukemic efficacy and to determine optimum dose and schedule of administration of ADC.

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Phase I Evaluation of a Biochemically Designed Combination: PALA, Thymidine, and 5-FU[1,2]

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Fifteen patients with advanced solid tumors participated in a phase I study of a biochemically designed combination chemotherapy program which employed PALA and thymidine (TdR) with 5-FU. PALA (250–2000 mg/m^2) was given 24 hours before a 90-minute iv infusion of TdR (45 g). 5-FU (100–150 mg/m^2) was given as a rapid iv injection 30 minutes after beginning the TdR infusion. This three-drug treatment was repeated once weekly for 3 weeks. Neurotoxicity, manifested as dizziness, lethargy, and confusion, was dose-limiting. Myelosuppression was noted at all dose levels, as was mild to moderate mucositis and diarrhea. Further clinical evaluation of this combination does not appear to be warranted. [Cancer Treat Rep 68:539–541, 1984]

Biochemical, or metabolic, modulation refers to the use of biochemically active compounds which alter the cellular pathways involved in the metabolism or action of a given drug in order to enhance the therapeutic index of that drug. Recent preclinical studies have demonstrated that the antineoplastic activity of 5-FU can be increased through metabolic modulation by pretreatment with thymidine (TdR) (1–3). Likewise, pretreatment with PALA, an inhibitor of de novo pyrimidine biosynthesis, increases the cytotoxicity of 5-FU against tumor cell lines in vitro (4,5), and enhances the antitumor effects of 5-FU in murine tumors (6,7). In the $CD8F_1$ murine mammary tumor, the three-drug combination of PALA + TdR + 5-FU produced better therapeutic results than either the combinations of PALA + 5-FU or TdR + 5-FU (3,8).

In clinical trials, therapeutic responses have been observed in patients resistant to 5-FU-containing combinations when these patients were treated with TdR prior to 5-FU (9). The clinical phase I trial reported here was designed to identify appropriate doses of PALA, TdR, and 5-FU for use in a biochemically designed combination chemotherapy regimen for patients with advanced solid tumors.

PATIENTS AND METHODS

Fifteen patients participated in this phase I study. All patients had pathologic proof of cancer, and all had tumors which were resistant to conventional therapeutic modalities. Each patient had a performance status of at least 50% (Karnofsky scale), an estimated life expectancy of at least 8 weeks, serum creatinine < 1.5 mg/dl, serum bilirubin < 1.5 mg/dl, a wbc count > 4000/mm^3, and a platelet count > 100,000/mm^3. No patient had received irradiation or chemotherapy for at least 4 weeks prior to beginning this study. There were seven men and eight women; the median age was 58 years (range, 33–73) and the median performance status was 70% (range, 50–90). All patients had progressed following prior chemotherapy, and all but one had received prior radiotherapy. Tumor types were as follows: lung, four patients; ovarian, three; breast, two; colon, two; sarcoma, two; mesothelioma, one; and thyroid, one.

PALA and TdR were supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. PALA was supplied in 10-ml ampules containing PALA disodium (1.0 g) with edetate disodium (1 mg) and sodium hydroxide to adjust the pH to 6.5–7.5.

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TdR was supplied in 500-ml bottles containing TdR (15 g) (30 mg/ml). 5-FU was purchased from Adria Laboratories, Inc, Columbus, OH, in 500-mg vials.

This trial was conducted in conjunction with a clinical pharmacologic study which was designed to identify a nontoxic, biochemically active dose of PALA. Initially, 2 g/m^2 of PALA was used; however, based on the results of that ongoing clinical pharmacologic study (10), the dose was reduced to 1 g/m^2 and then to 250 mg/m^2 during this phase I trial. PALA was administered 24 hours before TdR and 5-FU. The doses of TdR and 5-FU were selected on the basis of clinical and pharmacologic observations which have been previously reported (9), and the schedule was chosen to maximally block the degradation of 5-FU. TdR (45 g) was given as a 90-minute infusion. 5-FU was administered 30 minutes after the start of the TdR injection. This 2-day regimen of PALA, TdR, and 5-FU was repeated once weekly for 3 weeks. Four dose schedules were evaluated. A cbc, platelet count, and biochemical profile were repeated weekly during the 3 weeks of treatment and for 2 weeks thereafter. An evaluable cycle of therapy was defined as 3 weeks of treatment plus a 2-week period of observation.

RESULTS

Twelve of the 15 patients entered in this study were evaluable for toxic effects. One patient refused further therapy after the first week for logistic reasons; two patients died before completing a full cycle of treatment.

Mild to moderate myelosuppression was noted at all dose levels, although it was not dose-limiting; it was most profound in patients who had received extensive prior irradiation or chemotherapy.

The most prominent toxic effect was neurologic dysfunction. Almost all patients complained of lethargy, malaise, and anorexia beginning on the day of TdR and 5-FU administration. This was variable in severity, not clearly dose-related, and difficult to evaluate in the presence of progressive cancer. In addition to these nonspecific complaints, however, several patients experienced acute neurologic symptoms. These symptoms began during the TdR infusion, 15-30 minutes after the administration of 5-FU, and lasted several hours. Seven of the 12 evaluable patients, as well as an additional patient who died before completing a full 3-week course of therapy, complained of lightheadedness, imbalance, dizziness, and mild confusion. None of these patients had localizing neurologic findings, including nystagmus. This toxicity was reproducible with subsequent doses. In all patients but one, the complaints resolved completely within 24 hours, but one patient had dizziness which lasted for 4 months. None of these patients had evidence of CNS metastases, electrolyte imbalance, hepatic encephalopathy, or hypoxia.

One patient who received PALA (1 g/m^2) followed by TdR (45 g) and 5-FU (100 mg/m^2) became especially confused and agitated. Within 1 hour following completion of the TdR infusion, she developed an expressive aphasia which resolved in 4-6 hours; this did not recur with subsequent doses. The patient had taken one tablet of oxycodone (5 mg) with acetominophen (325 mg) (Percocet-5) during that first TdR infusion. The neurologic changes could not be attributed to metabolic abnormalities or CNS metastases.

Other toxic effects included mild to moderate nausea, diarrhea, mucositis, and rash. In addition, four patients experienced fluid retention after TdR infusion. Objective therapeutic responses were not observed.

DISCUSSION

Several schedules of TdR + 5-FU combination chemotherapy have been evaluated (9,11-14). Myelosuppression has been the major toxic effect, although neurologic toxicity has also been encountered (9,15). Both PALA (16) and TdR (17) alone can produce neurologic disturbances, but the neurotoxic effects of these agents differ from the clinical toxicity observed with PALA + TdR + 5-FU combination chemotherapy. However, 5-FU has produced changes in equilibrium and mentation similar to those exhibited by our patients (18). The precise biochemical basis of this toxicity is not well-established, but it is probably related to a metabolite rather than to 5-FU itself (19).

Because of the neurotoxicity observed, further clinical evaluation of this combination is not warranted.

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Methotrexate With Thymidine Protection or Rescue in Advanced Head and Neck Cancer: A Phase II Study[1]

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The usefulness of leucovorin in the reversal of methotrexate (MTX) toxicity has been shown in numerous clinical studies (1,2). It is well-known from experimental as well as clinical studies that MTX toxicity can also be reversed by thymidine (TdR) alone or in combination with a source of purine, as reviewed recently (3). In the treatment of advanced head and neck cancer, MTX is an effective drug with an overall response rate of 43% and minimal toxicity when given at moderate doses (100–1000 mg/m^2) followed by leucovorin rescue (4). The present phase II study was undertaken to investigate further the effectiveness of TdR in the control of MTX toxicity and to evaluate the clinical response of head and neck cancer to the combination of MTX and TdR.

PATIENTS AND METHODS

Initially, ten patients (six males; four females) with recurrent or metastatic squamous cell carcinoma of the head and neck were treated with MTX (600 mg/m^2 in a 24-hr infusion), with simultaneous or sequential infusion of TdR (8 g/m^2/day for 72 hrs). The median age was 59 years (range, 36–86). One patient had an Eastern Cooperative Oncology Group performance status (PS) of 3, four had a PS of 2, and five had a PS of 1. All patients had received prior radiotherapy, six patients had had surgery, and one patient had received cyclophosphamide but > 2 years previously.

Twelve patients (nine males; three females) were treated subsequently with MTX (600 mg/m^2 in a 24-hr infusion), with simultaneous or sequential infusion of TdR (1.5 g/m^2/day for 72 hrs). The median age in this group was 60 years (range, 46–75). Three patients had distant metastases; all others had recurrent or locally advanced disease. One patient had a PS of 3, six had a PS of 2, and five had a PS of 1. Eleven patients had received prior radiotherapy and five had had prior surgery. One patient had received a combination of MTX, cisplatin, and bleomycin (no response); another patient had received prior treatment with bleomycin and cisplatin.

MTX and TdR infusions were started simultaneously ("protection"); courses were given every 3 weeks in the first part of the study and every 2 weeks in the second part. If after two courses of simultaneous MTX and TdR infusion no effect on the tumor was observed, the 72-hr infusion of TdR was started at the end of the MTX infusion ("rescue"). If still no effect on the tumor was observed after another two cycles, the patient was removed from the study.

Blood cell counts were performed daily during drug infusion and at least once a week between courses. Blood chemistry was checked at least once a week. MTX and TdR plasma levels were monitored throughout each infusion by enzyme-inhibition assay and high-pressure liquid chromatography, respectively (5,6).

Standard criteria for response were used (7). Toxicity was graded according to World Health Organization recommendations (7).

RESULTS

In the first part of the study, the ten patients received a total of 28 courses of MTX-TdR, 21 with concurrent TdR and seven with TdR as rescue. Three patients had only one course, two because of grade 2 renal toxicity; one patient refused further treatment. Another patient refused further treatment after two courses and died within 4 weeks of progressive disease. No hematologic toxicity was recorded during or following 28 MTX-TdR infusions. Phlebitis was observed in 50% of the courses. Ten courses (three patients) were associated with mucositis, grade 3 in one patient and grade 2 in two others. Three courses were followed by transient grade 1 elevation of SGOT/SGPT. Nausea occurred in eight of 28 courses. No patient achieved partial or complete response.

In the second part of the study, 12 patients received 49 courses of MTX-TdR, 29 with TdR as protection and 20 with TdR as rescue; courses were repeated every 2 weeks. Two patients died, one shortly after the third course and

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TABLE 1.—No. of treatment courses associated with toxicity

Toxic effect	No. (%) of courses: MTX, 600 mg/m² TdR, 8 g/m²/day × 3 (total: 28 courses)	No. (%) of courses: MTX, 600 mg/m² TdR, 1.5 g/m²/day × 3 (total: 49 courses)	P value*
Hematologic	0	21 (43)	< 0.001
Stomatitis	10 (36)	15 (31)	NS
Nausea	8 (28)	11 (22)	NS
Phlebitis	14 (50)	1 (2)	< 0.001
Liver	3 (11)	2 (4)	NS
Cutaneous	0	3 (6)	NS
Renal	2 (7)	0	NS

*NS = not significant.

one after the fourth course of MTX-TdR. Both died of rapidly progressive disease without any signs of MTX toxicity. Toxicity was as follows: 21 courses were associated with episodes of hematologic toxicity, six of which were grade 3. Hematologic toxicity was the same during simultaneous and sequential administration of MTX and TdR. Other forms of toxicity were the same as those seen in the first part of the study and are summarized in table 1. In two patients a partial remission was achieved, four had no change, and six had progressive disease.

During MTX infusion, plasma MTX levels were 7.5 ± 3.2 (SD) μM. After the end of the infusion, normal decay curves for MTX were observed in all patients regardless of TdR dose. The pretreatment plasma TdR levels were 0.35 ± 0.17 (SD) μM. During infusion of TdR (8 g/m²/day), TdR plasma levels were fivefold to sixfold higher than the pretreatment levels (data not shown). TdR levels during infusion with 1.5 g/m²/day were not significantly different from pretreatment levels.

DISCUSSION

In this phase II study, the combination of MTX and TdR was given as first-line chemotherapy to all patients but two, in contrast with previous studies (8–12), in which the combination was tried in heavily pretreated patients, without substantial therapeutic effect. The results of this study show that concurrent or delayed infusion of TdR (8 g/m²/day for 72 hrs) prevented the development of serious toxicity from MTX (600 mg/m² in a 24-hr infusion) in patients with head and neck cancer. There was no indication of myelosuppression. However, grade 3 mucositis occurred in ten of 28 courses and nausea occurred in eight of 28 courses, indicating that TdR at this dose did not completely reverse all toxicity of MTX.

Prompted by the report of Howell et al (10) that the minimum dose of TdR required for (partial) protection against MTX toxicity in humans was approximately 1 g/m²/day and by the dismal therapeutic effect in our first ten patients, we continued the study but reduced the dose of TdR (1.5 g/m²/day for 72 hrs). We increased the frequency of treatment to every 2 weeks in a further attempt to improve the results. With this lower dose of TdR, substantial hematologic toxicity from MTX was observed, with a leukocyte count nadir of 1.1×10^9/L and a platelet count nadir of 38×10^9/L. It is unlikely that the increase in hematologic toxicity was due only to the more frequent MTX administration, since this schedule is generally tolerated without any problems with leucovorin rescue (2). Interestingly, the reduced dose of TdR was not associated with an increased incidence of mucositis or nausea (table 1), suggesting that these forms of toxicity are independent of plasma TdR concentrations but perhaps related to an antipurine effect of MTX. Phlebitis was a problem in 50% of courses in the first part of the study and was clearly related to the concentration of TdR (1.5% vs 0.3% in 0.6% NaCl), since it was seen only once in 49 courses of MTX with low-dose TdR.

Unfortunately, the reduction of the TdR dose was not accompanied by a significant increase in therapeutic effect, since only two partial remissions were seen in 12 patients, ten of whom had not had prior chemotherapy. A further decrease in TdR dose would lead to unacceptable toxicity (10).

The study was discontinued because this form of treatment appeared to be too complicated, requiring at least 4 days of hospitalization, and provided only minor antitumor effects.

In summary, 22 patients with advanced head and neck cancer received MTX at a dose of 600 mg/m² in 24 hrs concomitant with or followed by a 72-hr infusion of TdR at a dose of 8 g/m²/day in the first ten patients and 1.5 g/m²/day in the last 12 patients. No response or major toxicity was observed in the first ten patients, whereas two partial responses and substantial hematologic toxicity were seen with the lower dose of TdR. Considering the limited antitumor effect and the logistic problems (4 days of hospitalization per course), we cannot recommend this regimen for the treatment of patients with advanced head and neck cancer.

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Sequential Methotrexate Plus 5-FU in Advanced Breast and Colorectal Cancers: A Phase II Study[1,2]

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Fifty-two patients with advanced breast or colorectal cancer have been treated with methotrexate (MTX) (50 mg/m²) followed 6 hours later by 5-FU (600 mg/m²). The mean serum MTX level immediately prior to 5-FU administration was 1.37 μmols/L (1.37×10^{-6} M). Of 29 evaluable patients with breast cancer (six of whom had received prior chemotherapy), one achieved a complete response and five achieved a partial response (total response rate, 21%). Among 16 evaluable patients with colorectal cancer (three of whom had received prior chemotherapy), there were no objective responses. Although hematologic toxicity was generally mild, mucositis occurred in 20 patients (severe in three), and at least one early death was attributable to toxicity. These results indicate that at doses of MTX and 5-FU which are in general use in combination chemotherapy for breast cancer, sequential treatment does not have a therapeutic advantage. In colorectal cancer, the same combination is inactive. [Cancer Treat Rep 68:547–548, 1984]

Experimental studies using human carcinoma lines have suggested that sequential administration of methotrexate (MTX) and 5-FU may lead to drug synergy (1), and nonrandomized clinical studies in breast and colorectal cancer have been moderately encouraging (2–4). These studies have generally used an intermediate or high dose of MTX, requiring leucovorin rescue, and this increases the potential for toxicity. Lower doses of MTX may still achieve serum levels which might lead to enhanced 5-FU activity.

Thus, the first aim of this study was to examine, in breast cancer, the activity of sequential MTX plus 5-FU at doses conventionally used in "standard" protocols for this disease. The second aim was to assess the activity of the same regimen in advanced colorectal cancer in an attempt to enhance 5-FU activity without also increasing toxicity, as has occurred in some series (5).

PATIENTS AND METHODS

Fifty-two patients with advanced breast or colorectal cancer, all of whom had a creatinine clearance > 60 ml/minute, received MTX (50 mg/m²) as an iv bolus, followed 6 hours later by 5-FU (600 mg/m²) as an iv bolus; treatment was repeated every 2 weeks unless toxicity developed. Serum was obtained immediately prior to 5-FU administration from 21 patients during a total of 103 courses, and MTX assay was performed by the rapid homogeneous enzyme immunoassay method (EMIT, Syva).

Thirty-four patients had breast cancer (mean age, 54 years; mean Eastern Cooperative Oncology Group [ECOG] performance status, 1.3). Six patients had received prior chemotherapy (including MTX and 5-FU in five patients), and 30 had received prior endocrine therapy. The mean number of organ sites involved was 1.8, and most patients (ie, 26) had measurable soft tissue disease. Only four patients had liver metastases.

Eighteen patients had colorectal cancer (mean age, 53 years; mean ECOG performance status, 0.5). Three patients had received prior 5-FU. Most patients (ie, 13) had a single organ involvement, and the major site was liver (12 cases).

All patients who received at least two courses were con-

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sidered evaluable for response, although the mean number of courses given was 4.4, and the re-treatment interval was 2 weeks (as per protocol) in > 90% of courses.

Assessment of response was made clinically and with appropriate x-rays and isotope or ultrasound scans, with criteria for response in breast cancer being those described by the International Union Against Cancer (6).

RESULTS

Forty-five patients were evaluable for response. Reasons for inevaluability were early death (four patients, including one with evidence of sepsis and bleeding attributable to drug toxicity), patient refusals after a single course because of toxicity (two), and protocol violation (one).

Twenty-nine evaluable patients had breast cancer, six (21%) of whom achieved an objective response. One patient had a complete response, which continues at 6 months, in soft tissue deposits. The five partial responses occurred in soft tissue (four patients) and nodular lung deposits (one). In four of these patients the response lasted for 5 months. Three patients had disease stabilization lasting 4 months in one patient and 3 and 6 months in the other two. The remainder of the patients had progressive disease; eight of these have died 1–8 months following treatment.

Sixteen evaluable patients had colorectal cancer, and no objective responses were seen. Six patients had stable disease lasting from 2 to 11 months; the remainder had progressive disease, with three deaths to date.

Overall, chemotherapy was well-tolerated. Myelosuppression was mild; only eight patients had a total wbc count $< 3000/mm^3$, and only one had a platelet count $< 75{,}000/mm^3$. Mucositis was common but was minor (ECOG grade 1) in 20 patients and severe (ECOG grade 3) in only three patients. Nine patients had mild diarrhea, and three developed ECOG grade 1 alopecia.

The mean MTX serum level obtained immediately prior to 5-FU was 1.37 μmols/L (range, 0.21–3.60; median, 1.1). In six patients the level was below the assay detection limits. For the only two responders who had samples taken, mean MTX levels prior to 5-FU were 1.17 and 2.68 μmols/L.

DISCUSSION

The response rates reported in this study are lower than those observed in most previous trials of sequential MTX plus 5-FU, although previous negative studies in colorectal cancer have also been reported (7). This may relate to the choice of the lower dose of MTX, despite the observation that serum MTX levels were in the range which might have produced 5-FU enhancement.

In breast cancer the responses which did occur were disappointingly short, and the results in colorectal cancer were inferior to those obtained with single-agent therapy. We conclude that, with the schedule used, there is no evidence for any therapeutic advantage to sequential MTX plus 5-FU, and it is conceivable that higher doses of both drugs will be needed. Any further studies using these doses should probably include a time interval as long as 24 hours, as suggested by Kemeny and Michaelson (8), who thus obtained a partial response rate of 34% in colorectal cancer.

Such combinations may, however, lead to enhanced toxicity, and if future studies continue to suggest a therapeutic advantage, sequential MTX plus 5-FU should be subjected to a randomized comparison with nonsequential therapy to confirm that synergism does indeed occur clinically.

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Phase II Evaluation of Bleomycin in Patients With Advanced Epithelial Ovarian Cancer[1]

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Twenty-four patients received bleomycin twice weekly im. Treatment was given for 3 weeks initially, and if there was no evidence of disease progression treatment was given for another 3 weeks. Four patients had partial responses of 2, 4, 4, and 5 months' duration. Three patients had stable disease which did not progress for at least 4 months. There were ten patients with ascites; three of these showed a significant reduction in the amount of ascites present during treatment. There was a partial reduction of tumor in one of these patients. Toxicity was minimal with this regimen, and bleomycin deserves further study in the treatment of ovarian cancer. [Cancer Treat Rep 68:549–550, 1984]

Bleomycin has been shown to be active against many malignancies including lymphoma and teratoma (1–3). It is used in combination, and there have been reports of bleomycin, vinblastine, and cisplatin being effective against germ cell tumors of the ovary, with anecdotal reports of bleomycin being effective in patients with epithelial ovarian cancer (4,5). Recently, intracavitary bleomycin has been used in the control of malignant effusions (6). Some of the patients had epithelial ovarian cancer (7). We have studied the role of bleomycin in the treatment of ovarian cancer in patients who had had previous treatment with alkylating agents.

PATIENTS AND METHODS

Twenty-four patients with histologically proven epithelial ovarian cancer were entered in the study. All patients had had prior surgery and single alkylating-agent therapy and now had evidence of progression. All patients had tumors assessable either clinically or by radiologic means (normally, computerized tomographic scan). The presence of ascites, pleural effusion, or hepatomegaly was not regarded as evaluable disease. Patients were postmenarchal and only one was > 70 years of age. Patients were evaluated before treatment and after 6 weeks.

Bleomycin (15 mg im) was reconstituted in 2 ml of sterile water, with 0.2 ml of 0.2% lignocaine added to the solution. Treatment was given twice weekly for 3 weeks. If progression was seen, then therapy was stopped. Otherwise, treatment was given for a further 3 weeks (total bleomycin administered, 180 mg).

Response was assessed using World Health Organization criteria (8). The presence of ascites and pleural fluid was noted, and any change in the amount was monitored. Duration of response was assessed as the time of first evidence of response to the time that the disease was seen to be progressing.

RESULTS

Twenty-four patients have been studied. The characteristics of these patients are shown in table 1. All patients received treatment for the first 3 weeks. Seven patients progressed during the first 3 weeks and died before any reassessment could be carried out. Ten patients did not show any response and received a further 3 weeks of treatment. Following reassessment it was felt that these patients had not shown any response and had progressive disease.

Four patients had a partial response (17%); all four showed regression of abdominal masses. The responses were maintained for 2, 4, 4, and 5 months and were not related to pathology, grade, or stage of the tumor. Three patients had stable disease. The disease did not progress in these patients for at least 4 months.

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	24
Mean age in yrs (range): 60 (37-78)	
Histologic type	
Serous cystadenocarcinoma	11
Mucinous cystadenocarcinoma	3
Endometrioid carcinoma	3
Epithelial carcinoma (unclassified)	7
Grade	
Well-differentiated	6
Moderately differentiated	2
Poorly differentiated	7
Not specified	9
Signs of disease (pretreatment)	
Pelvic mass	8
Abdominal mass	12
Hepatic metastases	4
Ascites	10

There were ten patients with ascites. Three of these patients showed a significant reduction in the amount of ascites present during treatment. One patient also had a partial response of her tumor, but in the other two no tumor responses were seen.

Few side effects were associated with bleomycin, and no dose adjustments were necessary. All patients received the treatment as outpatients. There was no evidence of hematologic, lung, or gastrointestinal toxicity, with the exception of some mild nausea. Three patients had some pruritus and one of these patients noticed slight skin pigmentation. The most common side effect was fever, which eight patients experienced 2-4 hours after administration of the bleomycin. One patient developed herpes zoster during therapy but there were no other infective episodes.

DISCUSSION

This study was begun to determine whether bleomycin might be an effective agent in epithelial ovarian cancer. Some previous studies had shown control of effusions using intracavitary bleomycin (6,7). In this study, four patients (17%) showed objective responses and in an additional three patients there was no progression of the disease for at least 4 months. In three of ten patients with ascites there was good control of fluid accumulation with bleomycin. These results indicate that the drug has definite activity against ovarian cancer.

The drug was well-tolerated, with no evidence of hematologic or lung toxicity. In the eight patients who experienced fevers with the drug, this was a minor inconvenience and did not result in any dose modification.

This schedule of bleomycin has proved easy for both patients and staff. There is an undoubted response in epithelial ovarian cancer to bleomycin, and the drug deserves further study in this disease. At this center, bleomycin in combination with other agents is now being studied in a randomized clinical trial of chemotherapy for advanced ovarian cancer.

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Phase II Trial of PALA in Lymphoma: An Eastern Cooperative Oncology Group Study[1,2]

Franco M. Muggia,* Anastasios A. Tsiatis, Michael J. O'Connell, John H. Glick, Richard W. Opfell, and Abby Coren[2]

A phase II trial of PALA in malignant lymphoma was carried out by the Eastern Cooperative Oncology Group. The occurrence of hematologic toxicity in 17 of 35 patients in the study was noteworthy and was possibly related to prior therapy and/or marrow involvement. The finding of thromboembolic phenomena in four of five autopsied patients was also noteworthy. No antitumor activity was recorded. This experience discourages further trials of PALA in patients with malignant lymphoma. [Cancer Treat Rep 68:551–553, 1984]

PALA was tested for antitumor activity by the National Cancer Institute (NCI) after its characterization as a potent transition-state inhibitor of the enzyme aspartate transcarbamylase (1–3), a rate-limiting step in the de novo synthesis of pyrimidines. Preclinical evaluation indicated activity against Lewis lung carcinoma and other murine solid tumors but not against murine leukemias (3). This stimulated further interest in the compound, and widespread investigations were started late in 1977 under the aegis of the Division of Cancer Treatment, NCI, which labeled it a "high priority" compound.

Phase I studies reported the near absence of hematologic toxicity (4–9), except in patients with marrow involvement by tumor (5). Gastrointestinal toxicity, manifested by stomatitis and proctocolitis, and skin toxicity, including an intertriginous or generalized desquamative dermatitis, were dose-limiting in most schedules. Single-dose schedules were also complicated by common complaints of paresthesias and rarely by seizures or by subacute to more prolonged changes in mental status. The novel mechanism of action and relative lack of hematologic toxicity prompted the Eastern Cooperative Oncology Group (ECOG) to include PALA as a phase II agent for evaluation in patients with advanced, refractory malignant lymphoma.

The ECOG's phase II trial started in April 1980 with a dose schedule of 2.5 g/m^2 iv over 30 minutes daily × 2 days every 2 weeks. This split-dose schedule was used to avoid acute neurotoxicity. Eligible patients with wbc counts < 4000 and/or platelet counts < 100,000/mm^3 were assigned directly to PALA; otherwise, randomization to either PALA or amsacrine was carried out. To be eligible, patients had to have malignant lymphoma (material reviewed by the ECOG Pathology Panel), progressive measurable manifestations of disease, failure of no more than *two* previous drug combinations, and an ECOG performance status of 2 or better. Review of pathologic material by the Pathology Panel for lymphoma trials was mandatory.

The PALA arm was terminated in July 1981 after preliminary analysis disclosed no antitumor activity and considerable hematologic toxicity. Thirty-five patients (18 men and 17 women; median age, 57 years [range, 21–73]) were entered, of whom 31 are evaluable for re-

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[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: New York University Medical Center, New York (*F. M. Muggia* and *R. W. Opfell*, CA-16395); Dana-Farber Cancer Institute, Boston, MA (*A. A. Tsiatis* and *A. Coren*, CA-23318); Mayo Clinic, Rochester, MN (*M. J. O'Connell*, CA-13650); and Hospital of the University of Pennsylvania, Philadelphia (*J. H. Glick*, CA-15488). Other participating institutions include: Albany Medical College, NY (CA-06594); American Oncologic Hospital, Philadelphia, PA (CA-18281); Hahnemann Medical College, Philadelphia, PA (CA-13611); Jefferson Medical College, Philadelphia, PA (CA-14215); Johns Hopkins Oncology Center, Baltimore, MD (CA-16116); Medical College of Ohio, Toledo; University of Minnesota, Minneapolis (CA-20365); Northwestern University Medical Center, Chicago, IL (CA-17145); University of Ottawa, Ontario, Canada; University of Pittsburgh, PA (CA-18653); University of Rochester Cancer Center, NY (CA-11083); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-10948); University of Pretoria, South Africa (CA-21692); Tufts University, Walpole, MA (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, OK; and Wisconsin Clinical Cancer Center, Madison (CA-21076).

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sponse; the four patients deemed not evaluable died 1, 7, 10, and 11 days after the beginning of therapy and, in two instances, received only one-half of the first planned cycle of treatment. None of these patients had obvious complications of drug toxicity, and all four were considered to have died of rapid disease progression. Performance status at the outset had been 0-1 in 20 patients, and 2 in 15. Twelve patients had received one previous chemotherapeutic trial, and the remaining 23 had received two. Twenty patients had received prior radiation therapy. Bone marrow biopsy indicated lymphomatous involvement in nine of 18 patients in whom the procedure was done. Pretreatment leukopenia was present in one patient, thrombocytopenia in three patients, and both in one patient.

Results have indicated no significant therapeutic activity (table 1). All but four patients had progressive disease following one to four 2-day cycles of chemotherapy (mean, 1.9), and all but six have died. One patient with Hodgkin's disease involving the lung was considered to have stable disease throughout the six cycles of therapy but died of pneumonia 10 days after the last one in the absence of any hematologic toxicity. Two other patients had minor tumor regressions and one had disease classified as stable, but none were in the study > 4 weeks (two cycles) because of generalized deterioration in performance status; two of these patients had nodular lymphoma and one had diffuse large cell lymphoma.

Toxic manifestations (table 1) included common gastrointestinal and skin toxic effects and rare neurologic toxic effects similar to those previously reported. There was an unexpected finding of leukopenia and/or thrombocytopenia in 17 of the patients, only two of whom had pre-existing mild leukopenia (wbc count of 2800 cells/mm^3) or thrombocytopenia (platelet count of 97,000 cells/mm^3).

TABLE 1.—Results and toxicity for evaluable patients.

	No. of patients
Responses of patients with—	
Hodgkin's disease	0/8*
Diffuse large cell lymphoma	0/12*
Other diffuse lymphoma	0/7*
Nodular lymphoma	0/4*
Toxic effects	
Leukopenia†	12
Thrombocytopenia‡	11
Leukopenia and/or thrombocytopenia	17
Diarrhea	9
Mucositis	8
Skin rash	8§
Neurotoxicity	3¶
Allergy	1**

* No. of patients responding/No. of patients with disease.
†Wbc count (× 10^3 cells/mm^3): median, 2.6; range, 1.5-3.6.
‡Platelet count (× 10^3 cells/mm^3): median, 72; range, 24-96.
§1 severe desquamation.
¶1 seizure; 1 severe encephalopathy.
** Angioneurotic edema with laryngospasm.

One patient with pre-existing leukopenia (wbc count of 1400 cells/mm^3) and thrombocytopenia (platelet count of 8000 cells/mm^3) was excluded from hematologic toxicity assessment. Depression of granulocytes to 920/mm^3 associated with fever and possible pneumonia was documented in one instance. Platelet depression was marked (50,000/mm^3) in five instances, but usually recovered within 1 week. Prior irradiation or marrow involvement by lymphoma was not required for hematologic toxicity: nearly one-half of the patients with toxic effects had neither.

Of five patients who had autopsies shortly after the administration of PALA, four had evidence of thromboembolic phenomena. Thrombosis occurred in association with the compression of vena caval or iliac vessels in two patients. One of these two patients and one additional patient had recent pulmonary emboli. One other patient had an endomyocardial mural thrombus. Thromboembolic phenomena have been reported with another inhibitor of the de novo pyrimidine pathway, 6-azauridine triacetate (10).

In conclusion, the current experience discourages further exploration of PALA in the treatment of malignant lymphomas. Not only does PALA lack substantial antitumor activity, but it has greater myelosuppression in this group of patients than had been previously reported in phase I (4-9) or phase II trials (11-16).

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Vindesine in the Treatment of Advanced Colorectal Cancer: A Phase II Study[1]

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Vindesine, a semisynthetic derivative of vinca alkaloids, has proven to be active against diverse tumors in phase II trials (1). However, this effectiveness becomes minimal when vindesine is given to patients with advanced colorectal cancer who were previously treated with chemotherapy (2-4). On the other hand, the studies of Reynolds et al (5) suggest that vindesine is most effective in patients who had not previously been treated with chemotherapy, a hypothesis which has led us to test its use in a group of patients with advanced colorectal cancer who had not received prior chemotherapy.

MATERIALS AND METHODS

This study was conducted in 18 consecutive patients with histologically proven metastatic colorectal cancer with measurable disease. None of the patients had been previously treated with either chemotherapy or radiotherapy. The patients' characteristics are described in table 1.

The vindesine dose employed was 3 mg/m^2 iv on a weekly basis for 7 weeks, and then every 2 weeks until either the disease progressed or neurotoxicity was observed. A study was considered correct when patients received at least four doses. A 50% dose reduction was applied when leukocytes reached between 2500 and 4000/mm^3 and treatment was postponed when they were < 2500/mm^3.

Response evaluation criteria included: *complete response*—complete tumor disappearance for $\geq$ 2 months; *partial response*—a $\geq$ 50% reduction in the product of two perpendicular tumor diameters for $\geq$ 2 months; *stable disease*—no change or $<$ 50% reduction; and *progressive disease*.

RESULTS

Of the 18 patients studied, 16 were considered eligible for trial purposes. One patient was excluded because follow-up was not possible and another refused further treatment after two doses because of personal reasons (not related to toxicity).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Eligible	16
Median age in yrs (range): 60 (51-70)	
Karnofsky index (range): 80 (60-100)	
Sex	
Male	6
Female	10
Predominant metastatic site	
Liver	12
Lung	8
Pelvis	5
Response	
Complete	1 (6.2%)
Partial	0
Stable disease	8 (50%)
Progressive disease	7 (43.7%)
Toxicity	
Nausea	12 (75%)
Vomiting	6 (37.5%)
Leukopenia (cells/mm^3)	
2000-3999	12 (75%)
$<$ 2000	0
Paresthesias	15 (94%)
Reflexes	
Decreased	6 (37.5%)
Abolished	10 (62.5%)
Jaw pain	13 (81.2%)
Constipation	14 (87.5%)
Paralytic ileus	1 (6.2%)
Alopecia	15 (93.7%)

Of the 16 eligible patients, one died during the first month of therapy because of tumor progression (after four doses of vindesine), having been included in results analysis. One complete response was observed, which was evident by the disappearance of histologically proven liver metastasis by computerized tomographic scan and echography. This complete response prevailed for $>$ 4 months. No partial responses were observed.

The most important side effects were neurotoxicity (paresthesias, constipation, and jaw pain) and leukopenia (table 1).

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In conclusion, vindesine does not provide significant antitumor activity in advanced colorectal cancer.

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Phase II Trial of Vindesine in Adenocarcinoma of the Lung[1,2]

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The semisynthetic vinca alkaloid vindesine has been reported to have activity against non-small cell lung cancer, both as a single agent (1-4) and in combination with cisplatin (5). Because of this experience, we tested the drug in a phase II trial of 17 patients with adenocarcinoma of the lung with generally good performance status and no prior chemotherapy (table 1). We observed only one objective response in this group.

All of our patients had advanced, histologically documented adenocarcinoma of the lung. No patient had received prior chemotherapy, although one patient had had prior pulmonary surgery and five had had either prior or concomitant radiotherapy. All patients had measurable lung disease on chest x-ray, and all received pretreatment physical examination, SMA-12, SMA-6, and cbc with platelet count. Radionuclide and computerized tomographic scans were performed if indicated.

Patients received 3 mg/m^2 of vindesine (supplied by Eli Lilly and Co) weekly by rapid infusion through a well-running iv line. No patient received dose escalations, and dose reductions were performed for myelosuppression. Fifteen patients received a minimum of three doses; two patients were removed from the study with rapidly progressive disease after two courses. The median number of doses was four (range, two to 14 doses).

Seven patients developed neutropenia (< 1500 cells/mm^3), necessitating dose reduction in two and dose delays in four. Two of the seven patients with neutropenia had had prior radiotherapy. There were no neutropenia-related infections. Neurologic toxicity was seen in ten patients. One patient, who received concurrent narcotic analgesics and had intra-abdominal metastasis, had an ileus of 10 days' duration. One patient was removed from the study for severe, immobilizing leg weakness and significant peripheral paresthesias. Two patients developed mild peripheral paresthesias and six patients had loss of Achilles tendon reflexes.

One patient had a partial remission documented by a > 50% reduction in a lung tumor following ten courses of therapy. This remission continued until the patient's death (a non-treatment-related cardiac event) 9 weeks later. Seven patients had stable disease, and nine patients had progressive disease while receiving therapy.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 57 (36-65)	
Median performance status: 1*	
Sex	
Men	15
Women	2
Prior therapy	
Chemotherapy	0
Surgery	1
Radiotherapy	5
Site of measurable disease	
Lung	17
Sites of extrathoracic lesions (measurable and evaluable)	
Bone	5
Liver	3
Soft tissue	2
Adrenal	1
Objective responses	1†

*Symptomatic, ambulatory.
†Among 15 evaluable patients (7%). Confidence interval = 0%-25%.

Our study of 17 previously untreated patients does not confirm previously published (1-4) reports of vindesine's single-agent activity in lung adenocarcinoma, in contrast to a published report (5) of activity in combination with cisplatin.

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Phase II Trial of Vindesine in Advanced Squamous Cell Cancer of the Head and Neck[1,2]

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Vindesine, a derivative of vinblastine, is of interest in head and neck cancer chemotherapy because the parent compound (vinblastine) is thought to have some activity and because vindesine has activity against other squamous cell cancers (lung and esophagus).

PATIENTS AND METHODS

Eligibility was restricted to patients with the following: advanced squamous cell cancer of the head and neck which was recurrent after radiation therapy or metastatic below the clavicles; adequate marrow, liver, and kidney functions; Eastern Cooperative Oncology Group (ECOG) performance status of 0-3; and measurable tumor.

Of 31 patients from two institutions, six had therapy discontinued in the first 4 weeks, without clear-cut evidence of tumor progression, because of poor compliance or intercurrent medical problems.

Among the remaining 25 evaluable patients, 20 were male and five were female. Median age was 59 years (range, 42-87). Performance status on the ECOG scale was 0 in one patient, 1 in 14, 2 in nine, and 3 in one. Seventeen patients had had prior surgery, 24 had had prior radiation therapy, and 22 had had prior chemotherapy (with three or more drugs in 18 patients). The primary tumor was in the oral cavity in nine patients, in the larynx or epiglottis in seven, in the oropharynx in four, in the maxillary sinus in three, in the hypopharynx in one, and unknown in one. At the time of entry in the study, the primary site or the margins of resection were involved in 22 patients, the neck in 19; distant metastases were noted in six patients (four to lung and two to liver).

Vindesine (3 mg/m^2) was given iv every 7 days. Doses were reduced by 50% for wbc counts between 3000 and 4000/μl or platelet counts between 75,000 and 100,000/μl. Vindesine was withheld for counts below these limits and for neurologic toxicity impairing functions in daily life.

RESULTS

Two partial responses were observed among 25 evaluable patients, each occurring after 2 weeks of therapy. One 3-week response was observed among three patients without prior chemotherapy, in a male with a laryngeal primary who had neck nodes and liver metastases. The other response, lasting 3 months, was observed among the 22 patients who had had prior chemotherapy, in a male with a base of tongue primary who had lung metastases and had received four prior chemotherapeutic drugs in a single regimen. Unfortunately, the response was complicated by disabling peripheral neuropathy requiring the patient to use a cane and the physician to stop using vindesine treatment. Both responders had had prior radiation therapy; one had a performance status of 1, and the other had a performance status of 2.

Of the 23 nonresponding patients, six received two vindesine doses, seven received three doses, six received four doses, one each received five and six doses, and two received ten or more doses before being removed from the study because of disease progression. Median survival for the entire series was 3 months from the date of entry, with the two responding patients surviving 4 and 26 months (vindesine was stopped after relapse in each).

All but four of the patients had some degree of leukopenia, with wbc counts < 1000/mm^3 in two patients. One of these two patients developed pneumonia and recovered, and the other died of septicemia. In addition, five patients had wbc counts between 1000 and 2000/mm^3 and six between 2000 and 3000/mm^3. Thrombocytopenia was generally mild, with one patient each having a nadir platelet count between 25,000 and

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50,000 and between 75,000 and 100,000/mm^3. Two patients required packed rbc transfusions, and an additional seven had hemoglobin levels < 9.5 g/dl, but did not require transfusion. Peripheral sensory neuropathy was noted in nine patients, with accompanying hand weakness in two. Vomiting occurred in three patients, and one patient had a severe skin reaction at the site of paravenous drug infiltration.

DISCUSSION

This study demonstrates limited activity for vindesine against squamous cell cancer of the head and neck.

The results noted here are fully consistent with those of three other phase II trials of vindesine in this disease: three partial responses among 21 patients (two responses among 14 patients without prior chemotherapy) at Memorial (2), two partial responses among 14 patients without prior chemotherapy in San Antonio (3), and one complete and three partial responses among 16 patients treated at the Boston Veterans Administration Medical Center (4).

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Phase II Trial of Mitoguazone in Patients With Relapsed Small Cell Carcinoma of the Lung[1,2]

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Small cell carcinoma of the lung is highly responsive to polychemotherapy regimens (1). Once relapses occur, secondary responses are rare and the median time to death is 15 weeks (2). Mitoguazone (MGBG) is a synthetic polycarbonyl compound that acts as an inhibitor of polyamine biosynthesis (3). Because of its unique mechanism of action, lack of myelosuppression at maximally tolerated doses, and good patient tolerance on a weekly dose schedule (3,4), a phase II trial of MGBG was undertaken in previously treated patients at Memorial Sloan-Kettering Cancer Center.

Twenty-four patients, 14 males and ten females, with pathologically confirmed metastatic small cell carcinoma received MGBG. Patient characteristics of the 22 evaluable patients are shown in table 1. Twenty patients had measurable disease and two were considered to have one-dimensional evaluable (pleural and hilar masses) disease. All patients received prior chemotherapy with the Oat Cell V or VI regimen (5,6), and ten received radiation therapy to the mediastinum and primary complex. Nine patients relapsed from complete remission and three had not received chemotherapy within 3 months of starting MGBG.

The starting doses of MGBG were 500 mg/m^2 for poor-risk and 600 mg/m^2 for good-risk patients (Karnofsky performance status of ≤ 70 and > 70, respectively) and were administered as iv infusions over 90 minutes. One patient was treated with a dose of 400 mg/m^2. An increase or decrease of 100 mg/m^2 was performed, depending on toxicity. An adequate trial was defined as three weekly doses of MGBG. Standard phase II response criteria were employed. Two patients developed rapidly progressive disease after one and two

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total entered	24
Total evaluable	22
Male:female	12:10
Mean age in yrs (range): 58 (35-70)	
Median performance status (range): 70 (40-90)	
Prior therapy	
Chemotherapy	22
Radiation therapy	10
Indicator lesions	20
Lung	14
Node	5
Breast	1
Evaluable lesions	2
Starting dose (mg/m^2)	
600	8*
500	13†
400	1
Median No. of courses (range): 4 (3-7)	
Toxicity	
Median wbc count/mm^3 (range): 5000 (3800-6600)	
Median platelet count/mm^3 (range): 191,000 (52,000-252,000)	
Response	
Complete + partial	0
Progression	22

*Dose decreased for 2 patients.
†Dose decreased for 3 patients and increased for 3.

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doses of MGBG and were considered inevaluable for response.

Toxicity for this heavily pretreated population was acceptable. Myelosuppression was minimal. Only two patients developed platelet counts < 75,000/mm^3. Ten patients (46%) developed a decrease in hemoglobin > 1.8 g/100 ml (median, 1.5; range, 1.8–3.4). A myasthenic syndrome characterized by weakness and myalgias was observed in four patients (18%) and nausea and vomiting was observed in three (13%). Diarrhea and stomatitis were not seen.

No objective responses were seen in this patient population (95% confidence limits, 0%–11%), and the median survival was 10 weeks. Despite a mechanism of action that is different from other agents commonly employed, MGBG was an ineffective therapy for relapsed patients. The exact role of MGBG in an untreated population cannot be assessed from the present study.

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Phase II Trial of 4-Demethoxydaunorubicin in Patients With Non-Small Cell Lung Cancer[1]

Rudolf A. Joss,* Jean-Paul Obrecht, Pierre Alberto, Pierre Siegenthaler, Ançoise VanHelvoirt, and Franco Cavalli[2]

For the Swiss Group for Clinical Cancer Research (SAKK)

4-Demethoxydaunorubicin (Idarubicin, IMI-30) is a new daunorubicin analog which lacks the methoxy group at the C-4 position in the aglycone. The therapeutic ratio between antitumor doses and cardiotoxic doses evaluated in rats, mice, and rabbits is clearly in favor of IMI-30 (1,2). The phase I evaluation of IMI-30 within our group indicated that a dose of 15 mg/m^2 every 3-4 weeks could be safely given with predictable and reversible hematologic toxicity as the dose-limiting toxic effect (3).

Twenty-six patients with two- or one-dimensionally measurable, histologically documented, advanced non-small cell lung cancer were entered in this study. No patient was pretreated with anthracyclines or had a history of myocardial infarction, signs of congestive heart failure, or a clinically relevant arrhythmia. In doubtful cases, a cardiac ejection fraction had to be within normal limits. IMI-30 (15 mg/m^2) was administered by slow iv injection every 3 weeks. Treatment was delayed until full hematologic recovery had occurred. The dosage was reduced in subsequent courses by 25% if the leukocyte and/or platelet count nadirs in the previous course were between 1000-1999/mm^3 and 50,000-74,999/mm^3, respectively, and by 50% if the nadir counts were < 1000 and < 50,000/mm^3, respectively.

With three patients, it was too early for assessment. Three patients died early on Days 19, 20, and 21, two from unknown causes at home and one due to a myocardial infarction not felt to be related to IMI-30. Twenty patients were evaluable for response and toxicity (table 1). Seven patients had stable disease, for a median of 113 days (30+ to 162). Median survival was 97 days (19 to 259+). Myelosuppression, predominantly leukopenia, was the major toxic effect observed. Two patients with neutropenia required hospitalization after developing fever. Eight patients had moderate nausea and vomiting [Grades 1-2 according to World Health Organization criteria (4)], while 12 did not experience any gastrointestinal upset. Partial alopecia occurred in six patients. In two patients, cardiac abnormalities were observed after treatment with IMI-30. One patient developed bigeminy 8 hours after the second dose of IMI-30, which disappeared spontaneously after 15 minutes. A second patient

TABLE 1.—Patient characteristics, response, and toxicity

	No. of patients
Evaluable	20
Median age in yrs (range): 61 (51-70)	
Performance status*	
0-1	19
2-3	1
Prior weight loss	
< 5%	15
⩾ 5% or unknown	5
Histology	
Squamous cell carcinoma	7
Adenocarcinoma	9
Large cell anaplastic carcinoma	4
Extent of disease†	
Locoregional:extensive	7:13
Prior treatment	
Radiotherapy	2
Chemotherapy	2
Response to treatment	
Complete or partial	0
No change	7
Disease progression	13
Hematologic toxicity	
Leukocyte count nadir < 4000 cells/mm^3 ‡	17
Platelet count nadir < 100,000 cells/mm^3 §	4

* World Health Organization criteria (ref 4).

†Disease confined to 1 lung, including mediastinal and ipsilateral supraclavicular nodes, was classified as locoregional. Disease beyond these confines was classified as extensive.

‡Median (range): 2800 cells/mm^3 (500-39,300).

§Median (range): 184,000 cells/mm^3 (76,000-384,000).

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developed a paroxysmal supraventricular tachycardia 21 days after the second dose of IMI-30.

We have evaluated IMI-30 in a favorable group of patients with non-small cell lung cancer. Most patients were ambulatory, had not experienced marked weight loss prior to study entry, and had not been pretreated with prior chemotherapy. No patient experienced an objective tumor response. With no response among 20 fully evaluable patients, the predicted true response rate is $< 20\%$ ($P = 0.05$). We conclude that IMI-30, in the present dose and schedule, is an inactive agent in the treatment of non-small cell lung cancer.

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Recurrent Acute Toxic Optic Neuropathy Secondary to 5-FU

Since its introduction in the 1950s, 5-FU has been widely used as a chemotherapeutic agent. Visual disturbances generally have not been observed as a side effect of the drug. Here we report a case of recurrent, acute bilateral optic neuropathy probably secondary to 5-FU.

CASE REPORT

A 51-year-old woman was seen in October 1980 with a soft nodule on her mastectomy scar. In 1971, she had had a right radical mastectomy for breast cancer. All nodes were negative and no further treatment was given. Excisional biopsy of the soft nodule showed estrogen- and progesterone-receptor-negative recurrence of breast cancer. A bone scan showed extensive metastatic disease.

The patient began treatment with 5-FU, doxorubicin, and cyclophosphamide chemotherapy, as well as the antiestrogen tamoxifen. Tamoxifen was used when the patient volunteered to take part in an experimental trial of tamoxifen in patients with estrogen- and progesterone-receptor-negative breast cancer. The chemotherapy cycle was repeated every 4 weeks. 5-FU and doxorubicin were administered iv on Days 1 and 8, and oral cyclophosphamide was administered on Days 1-14. The tamoxifen was used daily throughout the study. The patient tolerated the initial cycles of chemotherapy without problems. Previously elevated alkaline phosphatase levels returned to normal.

In March 1981, during the fifth day of the fifth cycle of combination therapy, the patient complained of decreased vision in her left eye, headaches, and nausea. Ophthalmologic examination suggested mild bilateral papilledema. Visual field testing revealed a central scotoma and constricted peripheral vision. Over the next 2-3 days, the visual difficulty resolved. On the 11th day of the fifth cycle, 3 days after receiving 5-FU again, vision in both eyes became markedly decreased. She was unable to see 20/200 in either eye with her correction. The disc margins again appeared mildly blurred. There were no new complaints, and physical examination, including pupillary responses, was otherwise unremarkable. Laboratory studies were normal. A computerized tomographic scan of the brain, optic nerves, and chiasm was negative. Lumbar puncture and cerebrospinal fluid analysis were unremarkable. The patient was placed on prednisone (60 mg/day) and over a 2-3-week period, regained normal vision.

On the second day of the sixth cycle of combination therapy, the patient developed near-blindness in both eyes. Repeat history, examination, and laboratory work-up were unchanged. Repeat lumbar puncture was again entirely normal. A repeat bone scan showed dramatic improvement from the previous bone scan, with persistence of the tumor only in the sternum and possibly the spinous process of one cervical vertebra. Specifically, the skull showed resolution of all evidence of tumor metastasis. The EEG was mildly abnormal, with a sharp dysrhythmic quality in the background but no focal asymmetries. During this third episode of visual loss, the patient was restarted on prednisone as well as propranolol (for its possible vascular effects). Resolution of the third episode of impaired vision occurred over several months.

In reviewing the patient's history, it was noted that the three episodes of decreased vision occurred 5, 3, and 1 days, respectively, after her 5-FU and doxorubicin injections. Following the episode of the beginning of the sixth cycle in early April, all chemotherapy, as well as tamoxifen, was stopped for the remainder of April and part of May. In May, she was given doxorubicin without any visual changes. One month later, cyclophosphamide treatment was restarted without untoward effects. Subsequently, tamoxifen was given. She was not rechallenged with the 5-FU. Prior to her first episode of optic neuropathy, she had received a total of 6.8 g of 5-FU in eight doses over 4½ months.

In September, the patient's cancer broke through in the pleura and bone marrow, and chemotherapy was changed. Over the next 2 months she deteriorated rapidly, eventually dying of cardiopulmonary collapse. The 5-FU was never restarted and at no time did the patient complain of any more difficulty with her vision. She was alert until her death in November. No postmortem examination was performed.

DISCUSSION

Several cases of possible optic toxicity secondary to 5-FU have been reported. Ansell (1) reported the case of a woman who experienced partial blindness, which was thought to be secondary to macula degeneration or macula edema and possibly brought on by 5-FU therapy. Her sight improved when the 5-FU was discontinued and steroid therapy was initiated, but the pa-

tient died of a myocardial infarction before her vision returned to normal. Weiss et al (2) reported a case of optic neuritis seen in one patient. This patient's symptoms coincided with 5-FU therapy. Studies to exclude the possibility of tumor or another cause were not reported in either case. Other reported neurologic complications of 5-FU include an acute cerebellar syndrome after iv administration and myelopathy after intrathecal administrations (3).

In the present case, the patient had three episodes of optic neuropathy, each more severe and lasting longer than the preceding one. The episodes occurred 5, 3, and 1 days, respectively, after injections of 5-FU and doxorubicin. Tamoxifen, which is known to cause retinopathy (4) but not optic neuropathy without retinal lesions, was taken daily and had no such sequential relationship to the symptoms. Separate rechallenges with tamoxifen and all the chemotherapy agents except 5-FU caused no visual problems. There was no evidence that the patient had any active tumor at the time of her visual difficulty. Furthermore, subsequent carcinomatosis did not involve her vision. Vascular causes seemed unlikely, since the symptoms were gradual rather than stroke-like in onset and the central scotoma and return of vision were felt to be atypical for ischemic optic neuropathy.

The presumptive diagnosis was recurrent, acute, bilateral, toxic, optic neuropathy associated with the iv administration of 5-FU given as part of combination hormone and antiproliferative chemotherapy.

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Conventional Agents in Lung Cancer

The recently published article by Einhorn et al (1) comparing sequential versus combination chemotherapy with cyclophosphamide, doxorubicin, and methotrexate in patients with non-small cell lung cancer (NSCLC) raises several questions. The lack of a significant difference between the response rate to sequential therapy and to combination therapy is not unexpected in view of the relatively small number of patients in each arm. It should be noted, however, that the response rate to combination chemotherapy (22%) is more than twice the response rate to sequential single-agent chemotherapy (9%). This result in itself is not negligible for such a refractory disease as NSCLC. Patients should also have been stratified by cell type, since there is an imbalance in the distribution of patients with squamous cell and non-squamous cell carcinoma between the two treatment groups.

The statement by Einhorn that "there is no conclusive evidence that survival is prolonged with combination chemotherapy compared to single-agent chemotherapy or supportive care alone" (in NSCLC) is incorrect. A randomized trial by the Veterans Administration Lung Study Group (VALSG) has shown a significant advantage in survival for the combination of doxorubicin and cyclophosphamide compared to cyclophosphamide alone in squamous cell lung cancer (2). More recently, a randomized trial comparing a four-drug combination (methotrexate, doxorubicin, cyclophosphamide, and lomustine) versus placebo in NSCLC resulted in a significant overall survival advantage for patients receiving chemotherapy (3).

The remarkable survival advantage for objective responders and, to a lesser extent, for patients with stable disease compared to those who progressed is clearly seen in figure 3 of the article by Einhorn. The skepticism expressed by Einhorn in comparing survival data with VALSG historical controls is well-taken. According to Einhorn's results, however, it appears that the median survival of nonresponders was between 10 and 14 weeks. This is comparable to, or if anything, worse than, VALSG historical controls, if we can assume that the survival advantage for responding patients is directly related to the effect of chemotherapy rather than pretreatment characteristics. This could be verified by comparing survival differences for responders and nonresponders within each performance status category.

Finally, the same caution which is expressed by the authors in comparing different trials also applies to their own experience with vindesine alone, which produced a 28% objective response rate in 29 patients with NSCLC (4). In that trial, however, 22 patients (76%) had a Kar-

nofsky performance status of 80%–100% versus 44% in the present trial. Median survival of nonresponders was 24 weeks, compared to 10–14 weeks in the present trial. The prognostic differences between the patients included in these two trials may well explain the differences in results.

Everyone would agree that we need better treatments for patients with NSCLC. The majority of these patients, however, are not included in research protocols for many reasons. The benefits of chemotherapy with conventional agents for these patients should not be underestimated. The remarkable improvement in life expectancy seen in patients with stable disease or those with objective response (which applies to approximately one-half of the patients in the trial reported by Einhorn) should be emphasized rather than neglected and should be brought to the attention of their treating physicians.

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March 7, 1983

Reply

Most of the arguments that Dr. Chahinian makes regarding conventional agents have been very nicely refuted already in a previous issue of *Cancer Treatment Reports* in an editorial by Aisner and Hansen (1) concerning chemotherapy in non-small cell lung cancer. However, I would like to specifically comment on some of the points raised by Dr. Chahinian.

First, although there was a higher response rate to combination chemotherapy (22%) as opposed to single-agent chemotherapy (9%), this is not unexpected in this disease and these numbers were not statistically significant in the series of 104 patients treated. Second, Dr. Chahinian brings up the VALSG study showing a survival advantage of doxorubicin and cyclophosphamide compared to cyclophosphamide alone. This remains the one potentially positive randomized study showing any survival advantage, and it was very minimal. I would point out that Luedke et al (2) recently published a randomized study comparing single-agent vindesine versus the identical dosages of doxorubicin plus cyclophosphamide and found a very low response rate to doxorubicin plus cyclophosphamide and no evidence of any survival advantage with this combination regimen. The article by Cormier et al that Dr. Chahinian references regarding his own regimen of methotrexate, doxorubicin, cyclophosphamide, and lomustine (MACC) therapy also deserves comment. This study did show a survival advantage for MACC compared to placebo. This study has been used by our training program to educate fellows in the correct interpretation of the literture. Although this was supposedly a random prospective study, the survival was so brief with the placebo arm that there were obvious problems in stratification. There were a large number of early deaths from cancer in the placebo arm, although the protocol had eligibility criteria requiring an expected 6–8 weeks of survival. The nonresponders on the MACC regimen had a statistically significantly improved survival time compared to the nonresponders in the placebo arm, which also confirms major selection bias. The Eastern Cooperative Oncology Group (ECOG) repeated the MACC regimen (3) and had only a 12% response rate with median survival of 15 weeks, which is in marked contradistinction to Dr. Chahinian's study. This is not unique to the MACC regimen. ECOG (4) looked at eight different positive pilot studies with combination chemotherapy for non-small cell lung cancer and, in these 819 patients, the objective response rate ranged from from 13% to 24% with a median survival time, virtually identical in all arms, ranging from 18 weeks to 26 weeks.

The comment about responders living longer than nonresponders is ludicrous, has been put into perspective in the editorial by Aisner and Hansen (1), and doesn't deserve further comment. It is somewhat disturbing that this method of reporting studies in lung cancer continues

to deceive active authors.

In summary, we stand firm in our belief that, as yet, there is no "standard" chemotherapy for non-small cell lung cancer and it remains to be proven that there is any improvement in life expectancy for patients treated with chemotherapy. Thus, ongoing therapeutic research is a must and one is clearly justified in exploring all new treatments.

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August 8, 1983

Reply

We read with interest the letter to the editor by Dr. Chahinian regarding conventional agents and lung cancer, which finds fault with the statement by Einhorn et al regarding the lack of conclusive evidence supporting the benefit of combination chemotherapy treatments for non-small cell lung cancer (1). To warrant this conclusion, Dr. Chahinian has quoted two positive trials. One trial is a four-arm study which found a statistically significant survival difference between two of the arms (2). The other is a small trial which detected a marked difference in survival, despite its size (3). These two trials number among at least 11 trials which attempt to answer the same questions. The other nine trials are negative. Two positive and nine negative trials are not what one would consider "conclusive evidence" of benefit. One must conclude that the statement by Einhorn has validity. Furthermore, responder versus nonresponder survival comparisons do not speak to the issue of treatment benefit and are not pertinent to this discussion. We thus believe that Dr. Chahinian's statement "improvement in life expectancy" is not supported by any conclusive data.

Dr. Chahinian has summarized the available positive statements regarding chemotherapy for non-small cell lung cancer. Therapeutic nihilists should note that some benefit has been detected. However, the benefit is quantitatively too small to be routinely measured by clinical trials using survival as an end point, and thus, in our opinion, is quantitatively too small to justify widespread use of costly drugs in the routine treatment of advanced non-small cell lung cancer. Actually, Dr. Einhorn's group is to be commended for using a healthy population for new drug testing, where useful new agents are more likely to emerge.

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August 15, 1983

Progress and Controversies in Multiple Myeloma

Padua, Italy, April 12-13, 1984

The purpose of this international conference is to present and discuss the most recent progress in the biology and therapy of multiple myeloma. The program will include a limited number of free presentations, a poster session with ample time for discussion, and a commercial exhibition. The conference language is English and simultaneous translation will be available.

For further information, contact: Dr. A. Paccagnella, Divisione di Oncologia Medica, Ospedale Civile U.S.L., 21-35100 Padova, Italy (telephone: 049/661.011, ext 620; 635).

Seventy-Fifth Annual Meeting of the American Association for Cancer Research

Toronto, Ontario, Canada, May 9-12, 1984

The American Association for Cancer Research (AACR) will hold its scientific sessions at the Sheraton Centre and the Westin Hotel in Toronto. The program will consist of slide and poster sessions, several special lectures, and the Association's Annual Business Meeting. In addition, the Association will sponsor three symposia: "Growth Factors in Cancer," "New Approaches to Cancer Chemotherapy," and "Viruses in Human Malignancies and AIDS." The symposium on viruses will be co-sponsored by the American Society of Clinical Oncology, which will hold its twentieth annual meeting in the same locations on May 6-8, 1984.

Registration for the AACR meeting will take place in the Sheraton Centre beginning on the afternoon of May 8 and continuing throughout the meeting.

For further information, contact: American Association for Cancer Research, West Bldg, Rm 301, Temple University School of Medicine, Philadelphia, PA 19140 (telephone: 215-221-4565).

First European Conference on Advances in Antitumor Agents

Milan, Italy, May 30-June 1, 1984

This conference was organized by the Associazione Farmaceutici Industria and Consiglio Nazionale delle Ricerche-C.N.R., Progetto Finalizzato Chimica Fine e Secondaria. The meeting to be restricted to basic sciences (chemistry and biology) and will focus on critical re-examination of present approaches and proposals of new ones. The following topics will be dealt with: new antitumor agents of natural and synthetic origin; molecular interactions and metabolism; evaluation of screening programs; and peptides and hormones.

The text of the invited lectures will not be published in the form of symposium-proceedings in order to allow free and informal discussion and protect individual rights. A limited number of short papers will be considered for oral communication. In addition a poster session has been planned. The official languages of the conference will be English and Italian and simultaneous translation in these languages will be provided.

For more information, contact: Conference Secretariat, A. F. I., Via S. Tecla, 4-20122 Milan, Italy (telephone: Farmitalia-Carlo Erba, Milan, Italy).

Rational Therapeutics: A Seminar in Oncology

Duarte, California, June 2–3, 1984

This ninth annual symposium is sponsored by the Department of Pharmacy Services, City of Hope National Medical Center and will be held at the Anaheim Marriott Hotel in Anaheim, California. It is designed to provide members of the health care team with current and clinically useful information in the management of the cancer patient including the drug selection process, patient evaluation, and monitoring techniques.

This year's program will feature topics on: Empiric Antibiotic Therapy in the Immunocompromised Host, Nutritional Support in the Cancer Patient, VP-16 Chemotherapy, Cisplatin: 5 Years Later, Pharmacokinetic Studies of Anti-Cancer Therapy, Oncologic Emergencies, and Influence of Nutrition on the Development of Cancer. In addition, the symposium will have several clinical tutorials available covering specific areas.

The cost of this seminar is $120. Twelve hours of accreditation from either ACPE, California State Board of Pharmacy, or the California State Board of Nursing have been applied for. Physicians attending this course may report up to 12 hours of formal category I credit towards the California Medical Association Certificate of Continuing Medical Education.

For further information, contact: Debra Chung, Pharm D, Program Chairperson, Department of Pharmacy Services, City of Hope National Medical Center, 1500 E Duarte Rd, Duarte, CA 91010 (telephone: 818-359-8111 x 2247).

Epidemiology Summer Program

Chestnut Hill, MA, July 29–August 17, 1984

The New England Epidemiology Institute announces its summer program which will be hosted by the University of Massachusetts, and take place at the Amherst Campus in western Massachusetts. The program includes both methodologic and substantive courses, and will have classes for those seeking an introduction to modern epidemiologic concepts as well as those desiring a review of recent developments in epidemiologic thinking. Tentatively, 14 courses will be offered, including Epidemiologic Methods (introductory and advanced levels), Introductory Biostatistics, Intermediate Biostatistics, Modeling for Epidemiologic Research (Logistic Regression and Cox Survival), Cancer Epidemiology, Cardiovascular Disease Epidemiology, Nutritional Epidemiology, the Epidemiology of Reproductive and Congenital Disorders, Infectious Disease Epidemiology, Environmental and Occupational Epidemiology, Applications of Epidemiology to Health Services, Microcomputers in Epidemiologic Research, and Biomedical Writing. Invited faculty include excellent teachers and prominent researchers from leading universities. Registrants may receive credits toward graduate degrees, continuing medical education, or certification maintenance in industrial hygiene.

For more information, contact: New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

Fellowship in Medical Oncology, Hematology, or Combined Oncology-Hematology

July 1984 and July 1985

Applications are now being accepted for a fellowship program involving major teaching hospitals of Brown University's Program in Medicine, beginning July 1984 and July 1985. Clinical activities and opportunities for research are offered to qualified candidates with two years of approved medical residency and ABIM eligibility.

For further information, contact: F. J. Cummings, MD, Director, Division of Medical Oncology, or Manfred Steiner, MD, Director, Division of Hematology, Brown University, Box G, Providence, RI 02912.

Cancer Treatment Symposia: The Interdisciplinary Program for Radiation Oncology Research (Volume 1, 1984)

The Scientific Information Branch of the National Cancer Institute (NCI) is pleased to announce the availability of the third issue of *Cancer Treatment Symposia*: The Interdisciplinary Program for Radiation Oncology Research (Volume 1, 1984).

Sponsored by the NCI and the American College of Radiology, the Interdisciplinary Program represents the concentrated efforts and current thoughts of a large number of research scientists in many fields of radiation oncology; they have assessed the status of research and have made recommendations regarding possible future research in the areas of basic radiation physics, diagnostic oncologic imaging, treatment planning and delivery, basic radiobiology, tumor biology, predictive assays of radiocurability, dose fractionation and volume effects, sensitizers and protectors, combined modalities, radiation therapy and surgery, brachytherapy, radionuclides for systemic therapy, hyperthermia, particle radiation therapy, irradiation for immunosuppression, outcome analysis, and quality of life.

The Program was presented to the Board of Scientific Counselors, Division of Cancer Treatment, NCI, Bethesda, MD, on June 10, 1983.

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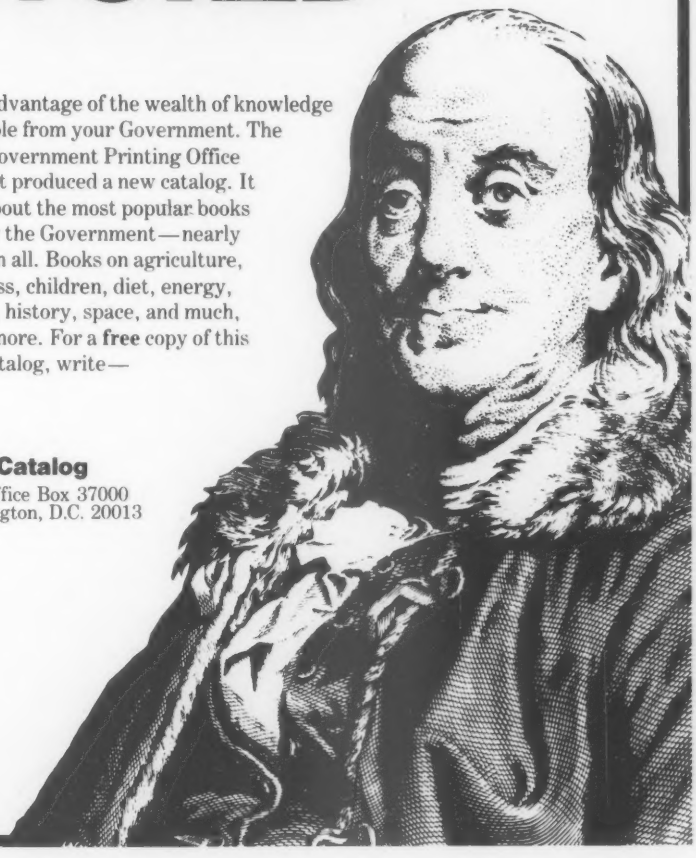

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